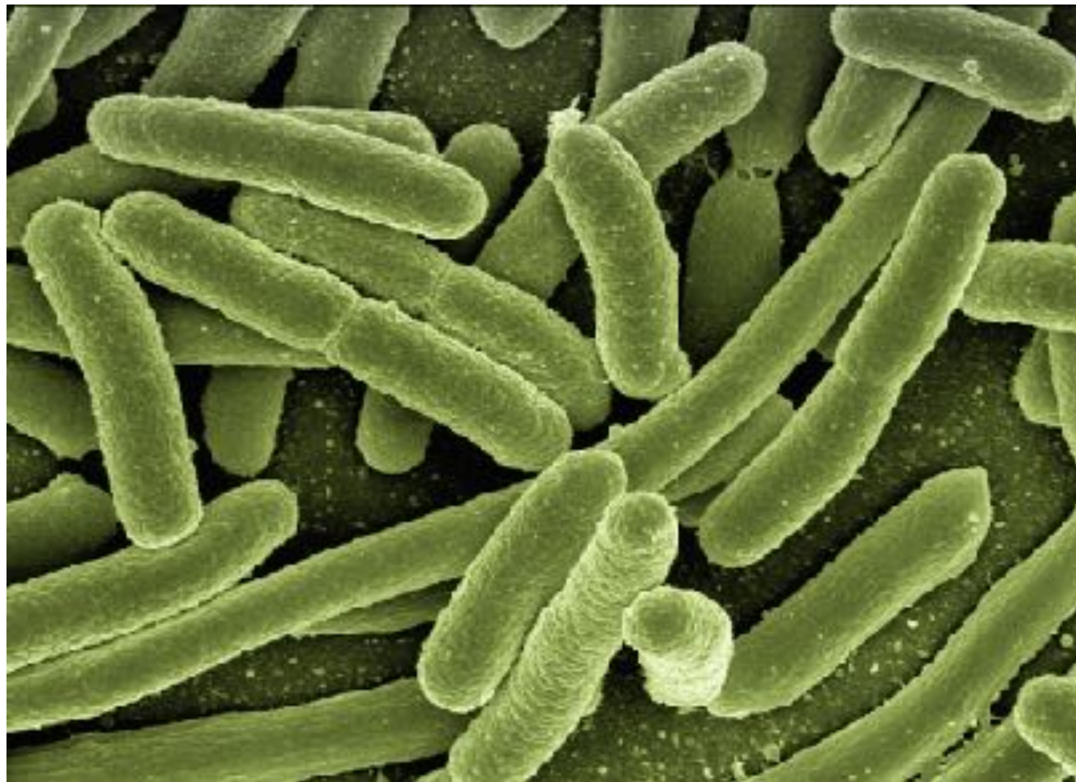
PMFList Yearbook

2010



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PREFACE

The PMFList (List) (a service of the Pharmaceutical Microbiology Forum (PMF)) provides a forum for communication among microbiologists involved in many industrial applications. This book (a “Yearbook” contains the vast majority of the questions posted to the List followed by answers provided by other List participants.

This specific Yearbook contains questions and answers from 2010. The questions and answers are listed in chronological order, meaning that the material from January comes first, and that from December is last. A consequence of this is that some of the questions posed in December have replies appearing in the 2011 book. All of the Yearbooks use the same format. The topic of the question posed (from the [OP]) is in

bold, centered font.

The question itself follows directly underneath the topic and is left justified in:

plain font.

Each answer as provided by the List participants follows, most often in the order received, with the answer preceded by

A#:

with numbers provided sequentially. Note that not every question received replies. Also, some questions posed late in the year are not responded to until the following year.

Basic spell checking was done and some minor editing to improve clarity. The name of the original poster ([OP]), and the names of respondents (often included in the provided answers) have largely been redacted. Many “pleases” and “thanks” were removed to shorten things up a bit. The participants in the PMFList are polite people, so it is best to assume questions often includes thanks for replies, and many replies also often included thanks. There is a lot of variation in the formats of the enclosed materials. This reflects the nature of the material as presented in the PMFList.

The hope is that these books will provide useful information, as well as a means of tracking how answers vary with each passing year. They will be provided to sponsors of the PMFList, with the quantity of books provided linked to the sponsorship level. They will also be provided to anyone else at the price of $49.95/book (send requests to David A. Porter at dporter@microbiologyforum.org). All books will be supplied in pdf format. Revisions of each Yearbook will be supplied to Sponsors/purchasers free of charge. Sponsorship details are at http://www.microbiologyforum.org/content/file/Sponsorship/PMF%20Sponsorship%20full%20form%202023%20September%2021%202023%20A.pdf. All proceeds go to support the PMF.

General caveat: the purpose of this, and all other such “yearbooks”, is to provide a consolidation of the questions and answers posed in a given year from the PMFList. The PMFList provides a forum for discussion of microbiology issues in the pharmaceutical and related industries. The information contained in this, and similar “yearbooks”, includes the professional opinions of individuals and does not represent the policies or operations of any corporation or government agency to which they may be associated. The PMFList is intended to serve as an open forum. The information in PMFList is solely for informational purposes. The questions and answers provided do not necessarily represent the views of the PMF board or regulatory agencies. The PMF cannot make any representations as to the accuracy or completeness of the information presented and the publisher cannot be held liable for errors.

Media overlay in aerobic MLT assessment

I have, I feel, a strange enquiry to which I'm hoping someone on the list maybe able to help me with. Recently it came to light that a supplier of ours performed their microbial limit test for aerobic micro-organisms using an overlay of media after an initial amount had been poured into the petri dish containing the material under investigation and allowed to set. The only thing we could think of was that the initial amount poured into the plate, as detailed in their method, was not quite sufficient to set. So why not just use more media and avoid the overlay?Is not the use of an overlay only of value to facultative anaerobes and generally reserved for more selective media like VRBGA rather than a general nutrient type as used in an MLT?Can anyone think of a reason why you would want to use an overlay when testing an excipient and how could you still call any count obtained 'aerobic'?
I look forward to your replies to this one.

A1: I've seen overlay of medium used to address spreaders. Have you asked them for their rationale?

A2: performing TAMC somebody do, on seeded SCDA, an overlay with melted agar 12%. The purpose is to reduce the colony final diameter.

A3: Overlay avoid spreading of colonies such as Bacillus and facilitate count.

A4: Especially for materials expected to have spreader-type colonies (gram positive rods, usually), an overlay procedure will help to avoid spreaders.

A5: We have a method for lactobacilli that requires the use of an overlay to (slightly) promote a "microaerophilic" environment when grown on APT (aerobic) agar. (non-selective, non differential)...apparently this gives the lactobacilli a little boost, but I have never seen that they really needed it in the first place.

I certainly wouldn't consider that it confers any serious anaerobic condition though; we run it in a blend with obligate anaerobes, and they don't ever present. The "microaerophilic" thang is only to reduce the oxygen levels slightly, (and I suspect very temporarily too!) and overlaying doesn't provide anything like the reduced oxygen/increased carbon dioxide of "proper' modified environment microaerophilic procedure.

Certainly, I'd still be happy calling an over-layed aerobically incubated plate “aerobic"
Hope this helps?

A6: I seem to remember a method of using overlay to reduce the occurrence of "spreader" growth. If a material is known to contain Bacillus for example that spreads to a point of not being able to accurately count, an overlay can be used to reduce the spreading affect and result in more distinct colonies.

A7: Previous to my current Pharma career, I worked in the food industry as technical and quality manager primarily looking after the microbiology lab - there it was industry standard to perform many pour plate techniques with an overlay. This, as rightly commented upon would not provide classic microaerophilic or anaerobic conditions but it did prevent the spreading of motile and sporulating microorganisms over the plates and obscuring other colonies or just making the counting process fairly challenging. Some organisms respond really well to the overlay - such as lactobacilli and Bifidobacteria. The overlay is also used for many anaerobic organisms - double-layer pour plate incubated in anaerobic conditions; this produces some fantastic, BIG (and very smelly) anaerobes.

The addition of an overlay also assists in colony formation and visually the colonies within the agar look pretty impressive in 3-D!

A8: I agree about the use of overlays - especially where automated plate readers are used for counting colonies, the overlay provides a far more consistent growth pattern which is readily countable - this being achieved by preventing spreading growth habits. (I must hasten to add that use of automated plate

readers should not normally be required in the Pharma environment, but my use was with samples from the public water supply!) The thickness of the agar does allow aerobic conditions - consider the rate of diffusion of oxygen into the medium compared with the rate of use by the organisms; however, the penetration of oxygen into bacterial colonies is a different matter!

I have obtained better recoveries of anaerobes when using overlays provided the plates are incubated in appropriate anaerobic conditions. I have only a small amount of data on oxygen concentrations at different depths of agar, but have found that use of visible anaerobic indicators demonstrates the (relatively) rapid penetration of the agar by the atmosphere in which a plate is incubated.

A9: i don't see any reason why one should use overlay technique rather that routine one.
Only one reason i can think about it to arrest the growth of swarming bacteria or rather reduce the swarming.

Identification of G. stearothermophilus

I would like your opinion on what is the current industry practice to qualify Geobacillus stearothermophilus either from ampoules or strips.

Currently we perform Catalase, Nitrate, VP test, Spores stain and starch hydrolysis test, once we grow the organism. Also what is the current practice

To perform VP and Nitrate test for this organism. Is there any other test anybody doing? Any help would be greatly appreciated.

A1: Refer to USP chapter on BI's and current USP

A2: An API 50 CH is easiest.

A3: My experience is to test in Vitek 2 Compact on BCL card. No extra tests are necessary.

A4: I assume you have an SOP requirement to use G. stearothermophilus probably for autoclave validations. However, you should be more concerned about the D value as one has very little to do with the other and you cannot determine a D value from biochemical ID tests. You probably want to do an ID test to assure what you are using is the organism you determined the D-value with or what grew out from a failed validation run. It is generally sufficient to tell if it is a G+ spore forming organism that is a thermophile.

A5: We use BioLog. Very easy and spot on with stearo.

A6: There are some new USP chapters on BIs. In the new chapters they state that the organism should ID as a Geobacillus sp. And in the new ones for items inoculated with spores they say the ID does not matter.

Cytotoxic drug bioburden

Can any one please tell me what will be effect of cytotoxic drug on cellulose nitrate filter if i am filtering a cytotoxic drug solution through a filter of cellulose nitrate during bioburden.

A1: Cytotoxic means the chemical (drug) has an effect on living cells. The term has no meaning as to any potential effect on non living materials such as a filter.

Pyrogen Free materials

I want to know if there is any special procedure should be followed for the sampling of pyrogen - free raw materials?

Interpretation of Results <61>

In USP <61>, the harmonization effort has added an interpretation of results section for TAMC and TYMC that essentially incorporates a factor of 2 of microbial recovery - i.e., 10e2 cfu - maximum acceptable count = 200. How is everyone implementing and/or utilizing this approach in terms of product release specifications?

Identification of micro-organisms found

in EP/USP Chapter "MICROBIOLOGICAL QUALITY OF NON-STERILE PHARMACEUTICAL PREPARATIONS AND SUBSTANCES FOR PHARMACEUTICAL USE" there is the following passage:

In addition to the micro-organisms listed in Table 5.1.4.-1, the significance of other micro-organisms recovered is evaluated in terms of:

* - use of the product: hazard varies according to the route of administration (eye, nose, respiratory tract);

* - nature of the product: its ability to support growth, the presence of adequate antimicrobial preservation;

* - method of application;

* - intended recipient: risk may differ for neonates, infants, the debilitated;

* - use of immunosuppressive agents, corticosteroids;

* - presence of disease, wounds, organ damage.

Where warranted, a risk-based assessment of the relevant factors is conducted by personnel with specialised training in microbiology and the interpretation of microbiological data. For raw materials, the assessment takes account of processing to which the product is subjected, the current technology of testing and the availability of materials of the desired quality.

My reading in the past was that, if you have found and identified a risky bug, that is not specific forbidden, you are anyhow not allowed to release this batch.

A written communication from a contract lab informed me, that from now on it is my duty to identify every single germ found during testing according to EP 5.1.4. (TAMC and TYMC as well) and to do a risk analysis (herbals excluded).

What's your opinion outside?

A1: Yes there are changes in the new Harmonised method, in the tests for Escherichia coli; Pseudomonas aeruginosa, Salmonella and Staphylococcus aureus, now further identification tests are required, previously, if the growth was not characteristic, the test could be stopped. In reality most labs identified all growth on the selective agars.

Depending on what your product category, then you need to decide as to whether you ID on TAMC and Y&M.

The tests for specified organisms will identify other organisms that may be clinically significant for your product. If your product has a low bioburden spec, then identifying your microflora will be good information for your facility, if you are prepared to do something with the data. If you have a product that has an indication for the immunocompromised, infants then you need to ensure that your testing can select for the "undesirables".

I am not aware from the harmonised method that all colonies need to be identified on TAMC and Y &M. It may have come up during a regulatory inspection on a product with a low bioburden requirements.

Also Herbals are not excluded from the need for risk assessment!!

A2: USP <62> reads that any growth on the plates for the tests for E. coli, P. aeruginosa, and C. albicans is confirmed by identification tests. For Salmonella, S. aureus and Clostridium the growth characteristic and/or confirming tests are described and if the isolated colonies match this pattern you confirm the identity.

In practice, most microbiologists identify any colonies.

Bacteria/Fungal cultures

I am updating a SOP for our environmental microbiology lab. There is a question about how many days a live bacteria/fungal culture is maintained before doing a transfer. What are other labs doing?

Limits environmental monitoring non sterile API production area

Regarding environmental monitoring are there any recommendation/ guidelines defining Alert and Action limits for a non sterile production area? In our area we do the chemical manufacturing of APIs that are suitable for the further processing to produce a sterile injection solution.

The area/ room is not classified (like a cleanroom A,B, C or D), but has different zones, including the core zone with the lamina, surrounding room and the lock for persons and materials.

Limits defined for the core and surrounding zone are Alert 100, Action 200 (cfu/plate). For the lock Alert 250 and Action 500.

These levels were done by my antecessor and nobody could tell on which basis they were defined. They existing limits seems to be rather high for me. Did I miss any recommendation / guideline?

In case not I would base the limits on the results I already got.

I would be great to get some ideas how other define those limits.

A1: I am not aware of API, but for formulation regulators expect classified area (100,000) for non sterile products as well & i read somewhere that it does apply for API also.

Anyhow one good idea can be to tighten the limits based on trends.

A1: You’re supposed to set your limits from historical data, but if you haven't got any then I guess that's no help. I work in a non-sterile pharmaceutical company and regularly test the production and packing areas of the factory.

The alert limits we currently use are, depending on area between 10 (for direct product contact points) and 150 (for contact points of thoroughfares). I inherited these figures but have found no reason to change them. Statistical data analysis suggests most areas would have roughly the figures set if I was to use standard deviation, Poisson, or 95/99th % rules.

Our action limit is also based on historical data but also incorporates the rule that if you surpass the alert limit on three consecutive occasions this will trigger an action limit.

Although I do not know your area requirements and the type of test you are looking to perform I will tell you that you will struggle to count greater than 100 colonies on a standard 55mm contact plate and you will also find that with an air sample any count greater than 200 on a 90mm agar plate is difficult to read with clarity.

You will also need to determine whether you will be looking for bacteria and yeasts and moulds separately or on the same plate or will use separate media for each.

The truth of the matter is that you set the limits because you have to justify them to an auditor. Remember environmental analysis is not a way to pin point a cause of contamination, but more for you to understand changes in the environment which could have detrimental effects on your product.

GMP and HACCP

What is the difference between HACCP and GMP? Is HACCP applicable to Pharmaceutical industry? Is there any standard/ regulation available for use of HACCP in Pharma industry?

A1: HACCP is a system/process for hazard (or risk) analysis and means of preventing same ie Hazard Analysis Critical Control Point. It has it roots in the chemical industry but was adopted by food manufacturers in the late 80's or thereabouts.

Pharmaceutical validation (IQ OQ PQ) processes have changed somewhat in recent years to encompass risk analysis and HACCP is one of the systems companies can use to enhance (or rationalise) the validation process. There are other risk assessment systems such as FMECA and PRA for example.

I guess risk assessment is now part of Good Manufacturing Practices (GMP) which are standard guidelines set out by the FDA to ensure drug development is carried out in safe and quality processes, to avoid contamination and ensure repeatability.

A2: HACCP stands for "Hazard Analysis Critical Control Points" and was begun in the food industry primarily as a means to identify and control points where contamination had occurred in food products. I'm sure that the pharmaceutical industry uses its own set of regulations, separate from HACCP. GMP stands for "Good Manufacturing Practices" and would include HACCP as part of these practices. I hope I answered correctly and I welcome further clarification from others.

A3: HACCP was originally developed by the American space agency NASA to ensure that food for astronauts was completely safe. It was derived from engineering concepts which focused on the quality of the product and all the components and the manufacturing process to eliminate any faults in the whole system. So in that respect HACCP is very similar to GMP in that it sits under the QA umbrella and is used to prevent mistakes in manufacturing. Just as we've seen GMP applied to the food industry even though originally developed by the pharmaceutical industry, the principles of HACCP can be incorporated into GMP but there is no regulatory pressure to do so. Hope that helps.

A4: HACCP is a tool for contamination control an cGMPs are regulations. Another way to put it is that the cGMPS are the "what" and HACCP is a how. The first official use of HACCP for pharmaceuticals is in the World Health organization WHO in 2003 Annex 7. The latest is in the EUcGMPs Annex 20 and ICH Q9 Quality Risk Management 2006.

A5: Basically HACCP was applicable to food industry only whereas GMP for pharmaceuticals. But now a days in risk based approach people are using HACCP as one of tool for process risk analysis in pharmaceuticals as well. You can refer FDA guidance on risk analysis.

A6: I agree with [redacted name]. HACCP is a great tool. Just remember that you must use HACCP as a tool and not as the entire quality system. Pharmaceutical and medical device companies have received Warning Letters from the FDA for following HACCP plans alone.

A7: I think that HACCP is required for Food processing industries, no require for Pharmaceutical industries. Because cGMP for pharmaceutical cover all types of risk eg. validation is a part of measurement of Critical control point. If cGMP guideline are followed by any pharmaceutical industry, HACCP in not required. Although WHO guideline are included HACCP in a chapter. I know each and every forum member are expert on cGMP on Pharmaceutical and each and every steps of GMP covers all aspects of drug manufacturing. It may be applicable for Active Pharmaceutical Ingredients(API) manufacturing. My opinion, Food safety is an important issue because food ingredients are from many biological natural origin and mineral sources. In Pharma cGMP, much more parameters, manufacturing steps are followed and validated. I think that cGMP is sufficient for drug manufacturing.

Establishing microbiological baseline

The FDA's 2004 aseptic guideline states that:
Characterization of recovered microorganisms provides vital information for the environmental monitoring program. Environmental isolates often correlate with the contaminants found in a media fill or product sterility testing failure, and the overall environmental picture provides valuable information for an investigation. Monitoring critical and immediately surrounding clean areas as well as personnel should include routine identification of microorganisms to the species (or, where appropriate, genus) level. In some cases, environmental trending data have revealed migration of microorganisms into the aseptic processing room from either uncontrolled or lesser controlled areas. Establishing an adequate program for differentiating microorganisms in the lesser-controlled environments, such as Class 100,000 (ISO 8), can often be instrumental in detecting such trends.

We use classified areas for:
*   Aseptic filling.
o   D/C airlocks leading to C (Class 100,000), and then C/B airlocks leading to the aseptic core, i.e. class B (Class 10,000) background to A (Class 100).
o   D/C airlocks leading to class C background to an isolator, class A.
*   Background to some of our API operations.
o   D/C airlocks leading to class C working areas.
*   Inoculation of fermentation and working with cell banks.
o   D/C airlocks leading to class C background to a class A UDF bench.

I would very much like to hear how various companies interpret FDA's guide to "routine identification of micro-organisms", i.e. how many organisms are identified and to what level in the different classified areas? Also how is this data used during trending and writing of the annual report that describes how the classified areas have performed in relation to their EM programs?

Formulation changes and <51>

I'm curious to know what everyone thinks the line is for a required AET <51> retest on a reformulation of a product. Does any formulation change require a requalification and retest for <51>? Or, can percentages of inactive ingredients be modified without requiring a <51> retest/requalification? I would be very interested to hear opinions on this. Also, please point me to the documentation that you may be drawing your argument from. I may have just missed it, but neither <51> or <1227> address the issue directly.

A1: This is going to be extremely formulation dependent. It has been my experience that even something as minor as changing a vendor for an excipient can affect the preservative system.

I would recommend putting an abbreviated PET in place:
 - reduced exposure times
 - two or three resistant organisms

Compare the performance of the marketed formulation (3-5 reps) with the proposed formulation (3-5 reps). Doing the test 3-5 times (different technicians?) allows you to have a bit more confidence that any difference between the formulations is real and not a product of the variance of the test. If it is deemed necessary, the full, official PET can be done after you are sure of the formulation performance.

In my opinion, failing to do some comparison of this type entails unacceptable risk, even if the formulator assures everyone that the formulation will be the same. Bugs don't listen to formulators.

A2: We always performed AET test on reformulations of products. Not sure if there is a guideline requiring it, but it is a good practice and policy. Even the order of how the ingredients are added during product manufacture can affect the antimicrobial properties. So any change in formulation is potentially likely to affect it.

A3: You can refer SUPAC guidance for changes in formulation.

A4: Any change of formulation should be investigated since seemingly insignificant modifications can have a significant effect on preservative efficacy performance.

A5: I don't know which guidance regulatory you use for your testing (USP, BP or EP) But according to USP regulation (I think the same with the others) any changes in product formulation required a testing requalification to evaluate the impact of the new ingredient(s) on MLT recovery. This is mean that may be other factors has to be changed in your testing: the sample preparation, dilutions, neutralizer, pH, etc Also, its very important to know the nature and the microbial bioburden of each new ingredient to determine the bioburden of the finished product.

antimicrobial agent for MLT

We are dealing with API products. We manufacture erythromycin and it's salts.While performing MLT we face problem with antibacterial property of the product and also in the growth promotion of the cultures given in the pharmacopoeia. Can anyone please help and suggest any suitable antimicrobial agent which we can add to neutralise the antibacterial property of the product so that we can get the exact results of the count without changing any other property of our product?

USP <62> Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms

Under the Interpretation section of Escherichia coli testing by USP <62>, growth of colonies indicates the possible presence of E.coli and is confirmed by identification tests. From a compliance standpoint, is there a list of identification tests for Escherichia coli that we can perform method verification on? Do we use biochemical tests defined in Microbiology texts, such as Bergey's Manual of Systematic Bacteriology? Is method validation necessary for methods written based on micro texts in order to meet regulatory compliance requirements? If so, what does validation entail - is it described somewhere in the USP like it is for validation of analytical procedures?

A1: We use API 20E (BioMerieux). Each lot of API has to be checked according to the instruction.

Harmonisation test, pharmacopeia and Government Inspectors

Well that covers about everything that we should be worrying about.

So the question is (derived from this extract).

We have implemented harmonized methods as interpreted from the BP/EP, as well as the changes in materials (don't get me started on Manitol Salt Agar again), all seemed to be going very well until recently (just now). It seems that our regulatory affairs department has been informed by the government inspectorate that testing products to the new pharmacopeia standards is all well and good EXCEPT........

where changes have indicated a change in test e.g. where the enterobacteriacea test was replaced by E.coli test the removal of the enterobacteriacea test must still be performed and can only be excluded if written justification is supplied.

Now I'm not one for writing hugely pointless reports, would much prefer to point at the pharmacopeia and say "is that good enough", or even better stop testing for E.coli altogether using the current methods and just use the VRBA plates that I use for ents testing (1 in 1000 +ve results can be subsequently tested for E.coli).

Has anybody else (ever moaned as much about microbiology?) had this problem regarding continued testing of old methods or is it something that I should have either a, foreseen and continued or b, just wrote the report.

In the case of b, does anybody have a report they would like to share ;)

Thanks for

a, reading
b, understanding
c, sending said report
d, being a government official and saying "don't worry about it, the ents test is more than enough of a tool to show the absence of E.coli"
e, being anyone in the field of microbiology that agrees that Manitol salt Agar sucks.

A1: You are obviously dealing with a government official(s). If you had SOPs that specified certain testing requirements and specifications and did not just reference compendial standards/methods then they are correct. You are, in fact, changing your requirements/ test methods which usually requires justification. The usual approach is to show that what you have changed to or propose to change to is equal to or better than what you propose to change from. That will usually satisfy officialdom.

Cleanroom photos/images

Does anyone know of a source for images/photos of cleanrooms and/or manufacturing for pharmaceutical, biotech, MD&D?

I am interested in paying for the rights to use the images.

I have looked at getty images and found very little.

A1: Check out iStock Photo, at www.istockphoto.com/index.php. Small resolution images are reasonably priced, and they do have a good selection of biotech.

neutralization in mlt

I'm curious what others have done/do for neutralization for Salmonella testing in Microbial Limits other than increasing the dilution or adding tween and why (what is the reason your neutralization method works or would be hypothesized to work)?

Also how does adding tween to a 100% water soluble TSB sample prep act as a neutralizer?

A1: It's typically considered that Tweens mitigate antimicrobial activity through partitioning of preservatives into micelles and I think the levels used are well above CMC. (see: http://www.springerlink.com/content/9050007t7253h974/)

Data supporting this were based on experiments with more classic antimicrobials (BAC, parabens). For organic acids, the pH is a confounding factor with the dissociated acid having a lower affinity. So not only dies the higher pH of medium minimize efficacy of what's soluble - it also facilitates partitioning. These are derived from measurement of simple systems and some folks have descried increased preservative efficacy in the presence of Tweens.

A2: The neutralization scheme often has more to do with the test sample matrix than a particular organism. While there will obviously be different impacts on different organisms, I have often found a lack of really good literature for neutralizers that actually work in a typical microbiology lab when looking at specific organism.

Growth Promotion of Water Test Media

I have a query for which I would be glad to hear the views of other forum members.

What would be the normally considered the standard Growth Promotion strains used for Plate Count Agar when used for TVAC of PW water as per APHA (SMWW)
I'm aware that the EP recommends B. subtilis (spizizenii) ATCC 6633 and P.aeruginosa ATCC 9027 for R2A and had hoped that a similarly reduced listing would apply to PCA in the specific case it was used for water testing at 30-35oC.

Currently, I am falling back on recommending the standard battery of tests, S.aureus, P.aeruginosa, B. subtilis, C.albicans and A. brasiliensis, normally applied to TSA as per <61>, as it has come to my attention that some inspectors are looking to see all these even if the PCA is only being used for testing Purified Water and 20 -25oC incubation suitable for Yeasts and Molds is not being employed. Interestingly, the same inspectors did not include E.coli, though I suppose if the rationale was to include all major potential contaminant types, P.aeruginosa would adequately cover the Gram-negative side of things (or would it?). Anyway, I would like to hear the ideas of others within the sector.

A1: First we should remember that the purpose of growth promotion testing is to confirm that the R2A medium has been correctly prepared and shows lot-to-lot consistency.

For this QC function we can select cultures appropriate for the medium as recommended by the AWWA/APHA standard methods, the compendia and the media manufacturers.

The selection should consider the type of bacteria that may be found in your water system as well as the incubation conditions.

Given that most companies are using the Ph Eur requirement of 5 days at 30-35 degree C B.subtilis (spizizenii) ATCC 6633 and P.aeruginosa ATCC 9027 plus an environmental isolate like R. picketti may be a good choice.

Passages for Positive Control Work

I have another query for which I would be glad to hear the views of other forum members

In positive control testing, are all discrete incubation steps viewed as a passage. I'm familiar with the seed lot approach but have encountered two
differing views with regard to positive control testing and how manipulations within the final test sequence are considered.
As a particular example let us take the "Tube Test" for Coagulase.
When tubes are read a second time at 24 hrs for the presence / absence of clotting, is it required that this culture have passed no more than 5 passages at the time of reading the test. i.e. the inoculum used to seed the reaction tube could not have been more than 4 passages. Similarly, plating out turbid broths for Growth Promotion or Positive Control tubes onto agar plates for confirmation, the original inocula could be no more than 4 passages and the final stage a maximum of 5 passages.

Obviously this also applies to suitability testing. Some I have encountered view that the initial test inoculum must be no more than 5 passages from the type culture but that subsequent manipulations / subcultures within the actual test sequence do not get counted or otherwise multiple sequence tests e.g. those employing pre-enrichment, selective enrichment and two phase confirmation steps, such as certain Salmonella protocols, would often require official freeze dried preparations for control or suitability testing purposes unless the consecutive steps were tested individually with new inocula, which is not necessarily a true reflection of the overall process. I can see their point but I would have thought all sub-culture steps are passages. Any views?

A1: Sounds like something that an anal-retentive inspector would try to make a fuss about. Other than that I wonder if it's worth worrying about. If your original fifth-passage inoculum works, use it and don't worry about it. If it seems that the culture is getting weak (it does happen) then try a lower passage (is that correct terminology?) inoculum.

Specified Organism Suitability - shortest incubation

I am in the process of writing an SOP for USP <62> testing of our products (API's) by an outside contract lab and need to know how others have handled the problem of Suitability incubation times. The chapter indicates under Suitability of the Test Method to "Perform the
test as described under Testing of Products using the shortest incubation period prescribed."

I understand that this is due to the fact that lab organisms are relatively healthy when compared to those found in product samples and if the method is appropriately designed, recovery at the shortest incubation should be easily achieved and ensure recovery of more stressed organisms using somewhat longer incubations. However, considering the number of 18 hr incubations involved in the Tests for Specified Microorganisms procedures, how does a non-24 hr/day facility meet this requirement?

For instance, Salmonella has three back-to-back 18 hour incubations. Even if the first was begun late in the day, the second would end in the middle of the night. This creates a problem for labs with operating hours of 8 AM to 5 PM.

I would appreciate any advice you can give me on this issue.

A1: We do follow the minimum incubation times for suitability testing. We get around the "off hours" by making sure that the plates/enrichments don't go back into the incubator until after a specific time in the day to allow for reasonable times to pull the next step. We then pull the broth/plate and do allow it to go into refrigeration prior to processing the next step.

A2: you should validate a method to show that amending your incubation time to 24 hours does not have a negative effect on the test method or recoverability of the organism.

A3: We established our minimum time at 21 hours per an SOP and write the test methods to follow.

A4: You may want to validate refrigeration of sample at such times during suitability. Demonstrate during suitability that continued testing from a refrigerated sample does not interfere with recovery of low bioburden (less than 100 CFU).

Note the use of Refrigerator in appropriate documents.

A5: Connie- we wrote internal methods and changed the minimum incubation time from 18 hrs to 20 hrs for most of the tests in <62>. We then performed our suitability testing using the 20hr incubation. S. aureus was the only trouble-maker and required 24 hours in TSB, then 20hrs on MSA.

A6: It seems that refrigeration until 24 hours is the consensus.

Could you tell me how to go about setting up a validation for refrigeration?

- Would it have to be done with every organism and every type of media involved?
- Would it have to be done in the presence of product and, if so, for every product?
- If not, would documenting the appearance of the Peptone Group for each enrichment and each incubation step at 18 hrs and 24 hrs (with refrigeration) be sufficient?

A7: Thanks for the reply!

I actually did just what you suggested while I was waiting for replies, but using 21 hours.

Why did Staph have to go to 24 hrs? Was it just due to the product or were there issues with the Peptone Group, too?

First raw material

I'm interesting to know how can I establish microbial limit of first raw materials?

missed environmental data

we produce aseptically filled injectables.

During the setup activities before to start filling, the operator missed
to monitor his gloves.

I'd like to know how such a deviation could be managed.

Should we reject the batch, maybe ?

A1: The event you describe is definitely classifiable as "lack of sterility assurance"- only slightly ameliorated by the fact that it was a setup operator and not an operator involved in the actual fill. You have a few critical questions to ask yourselves, and you need to balance these answers against the potential cost of dumping or re-processing (if allowed) a batch of product. Not an easy situation.

Were other operators involved in the setup, and were they monitored?
Results?
Were all operators involved in the FILL monitored? Results?
Are environmental samples (RODAC's, swabs) taken on the equipment post-fill? Results? And lastly: Any initial product sterility samples (first samples out of filling needles, perhaps)? results?

You can perhaps counterbalance this omission with existing data- as long as you have a preponderance of information- but you do have a critical gap in your monitoring data there. Whatever your decision, document everything, including the decision-making process and the information used to make it, very carefully- you may well have a need to walk highly-suspicious investigators through the process at some point. The CLEAREST and 'easiest' answer is simple: Lack of sterility assurance means you cannot in good conscience release this batch of product. We all know what the sterility test on final product is worth- aseptic operations are based on the sterility ASSURANCE of the process, not the final test results, and you've got a critical hole in that assurance.

A2: I have never heard of a batch being rejected because of an EM failure (especially not an individual missed sample). A deviation will obviously have to be filed, but a simple risk assessment should be able to determine whether or not the sterility of the batch was compromised. Do you sample the fill line after the batch? Do you have data on that individual during the course of the batch? Do you have trending that would suggest that individuals are often "dirty" during the setup period? There should be plenty of past data (and future data) to leverage that the batch is fine. Obviously I would also include some sort of CAPA to ensure this does not happen in the future.

A3: Few questions to ask.

What would be the response if the analyst had growth on the gloves?

What is the operators history (trend)?

We have had these instances before for various samples - personnel, air, surfaces. Our response has been to trend the results for that sample as well as additional samples that were related to the fill.

As an example - missing active air site - we would look at that sites historical trend as well as the passive air sampling in the same area and additional active air samples taken before and after the missing one. All of this would be recorded in a non-conformance document and a risk assessment performed for the product filled in relation to the data that was gathered.

In short - it is dependent on the trending of that employee and additional EM data from the fill to determine the risk to the filled product. I would not summarily reject the product for one missing sample.

A4: Presumably this is only a single piece of missing data from a larger, satisfactory data set. If all other EM data around the batch was okay and historical results for this operator during this type of operation have always been satisfactory then I would think you can use these to justify this single piece of missing data and allow the batch to be accepted. However, if you have further missing data or OOS results during the batch this will make it more difficult to justify the missing data.

A5: I think you want to avoid rejecting batches of product.
Set-up is not as crucial because there is usually no critical or aseptic operation occurring.
The operators usually change gloves after setup also so the gloves may not have been used during the fill operation.

Having a deviation that is well written with a well thought out CAPA may be the way to go. Expect that the FDA will review everything document.

Question: why was QC Microbiology not in the room responsible for performing the personnel plating as part of the Environmental monitoring?
QC Microbiology personnel should be doing the personnel plating and EM in the room. The operator has a lot to do and may forget stuff if they are doing other persons functions. Also there is a question of integrity if people plate themselves.

The main focus should be the CAPA, how are you going to prevent this from happening and also the risk assessment/investigation of how this impacts the product. If no product was open and the operator changed gloves the potential impact may be low.

I would break down the Set up process and rate as low to high risk to product sterility. As a last resort maybe double the vials used for sterility testing.

Was any of the EM data captured later bad or unusual?

A6: Environmental monitoring and personnel monitoring are part of the overall Quality control of the product. Some other parts for it are bubble point at the end of the fill, bioburden of the material prior to fill, sterility test results.

I would try to do risk assessment before getting to the point that batch should be discarded:
Does this operator have gowning qualification
Does this operator have a history of recoveries on the gloves
Does your QA unit observe product fill and had some observations for this batch.
Were other environmental samples taken during the fill and want were the counts.
Do you require to have double gloves when the unit is being set up
Was it the same operator performing the fill and were the gloves monitored at the end

Some other questions can come along during your investigation before you consider to discard the batch.

A6: This should be under the heading of microbiology humour. Go and tell your Production Director that you want to reject a batch because an operator didn't record the testing of his gloves and you'll understand what I mean.

A7: Interesting question....I don't have much experience with injectables, but have some thoughts.

I would first ask if your SOP has this covered already...I'm guessing it doesn't, but probably will in the future.

I'm assuming that the standard practice would be for the operator to wear the appropriate sterile gear and then do some touch checks on gloves and such for microbiological/environmental monitoring and then proceed with the work to be done. Plates would be incubated and 3-4 days later you would have results from the plates.

- what happens to the batch if these plates show colonies?
- would you reject a batch that tested clean if the environmental data showed colonies?

What activities was the operator involved with (i.e., risk to product contamination)....if he was mopping the floor, I would guess minimal, if he was adjusting fill nozzles, then greater risk).

A8: One glove sample being missed is not enough to reject a batch. Of course you will need to do a full investigation. You sterility QC results of the filled product should support (not be the only supportive data for) your decision to reject or accept the batch. From an investigational point of view, you should consider all the other monitoring data available i.e. HVAC, EM results during the fill and the operator's history of clean room practices (either missing samples and/or behavior if trended). You can also look at it this way as well, if you found only the operator's glove sample that was contaminated during a routine set up, would you reject the batch based on this one glove sample?

A9: In case of growth, the batch should be rejected
Operators history is good (i.e. no OOS on the last year)

A10: I have to respectfully disagree. Remember, EM data are LIMITS, not SPECIFICATIONS. In an ISO 5 (Class 100) area, you are allowed 1 CFU on a settling or air sampling plate; you can apply the same criterion to a glove.

If you find more than than one CFU then you should conduct a comprehensive investigation and invoke your corporate action plan. Sterile fills merit the most scrutiny of any activity in the industry, but it's also important to apply scientific logic and empirical data to any decision that is made.

As you're heard me rant in the past, let science be your guide.

Designing a Microbiology lab

My company has decided to build a new microbiology lab and they ask me ideas for design it.

Can you share me your experience about it (work flow, optimal capacity, layout plan, etc.) or where i can look for information.

A1: One thing I have found invaluable in a lab is to have separate cold rooms and ambient storage areas for "quarantined" items (materials that haven't been QC'd yet). That way any new lots of media that are shipped to the site can be stored there, and after growth promotion and release they can be moved to the other cold room/storage room for technicians to access. I'm also a big fan of having a standalone

refrigerator for "contaminated" samples (i.e. plates for identification, QC cultures, etc.) so you do not risk placing contaminated media and sterile media in the same area.

A2: Separation of bacteriology and mycology work is very important. If you can, separate labs are best.

A4: Having enough hoods to have separate areas for clean and dirty work is a must. Better to have separate rooms but if you can't at least separate hoods.

Media and supplies in a microbiology lab are bulky, plenty of well placed storage so technicians are not forced to carry or stack trays of media is really helpful too.

To expand on the separate space for mycology, a small glove box is great for both containment of the samples and for operator safety.

Keep sinks and water baths as far away from testing areas as is feasible.

A steamer to melt down agar is a nice addition too if you are going to do pour plates at all.

Purchase and validation of incubators that can go into refrigeration on a timer is helpful especially if doing MLT work which requires some very specific incubation times that can be difficult for a 5 day a week lab.

A4: I just spent 2.5 years in this process. I am preparing a lessons learned article on it. Think carefully about what your work process is, what kinds of samples you will be handling, sample and work flow, laboratory access, storage needs, safety concerns, your own corporate and local or federal construction requirements, etc. Consider regulatory expectations such as, as already mentioned by a previous responder, separating quarantined and approved media and reagents, separate refrigerators for contaminated plates, separation of dirty and clean operations, etc. Overestimate the space you need to do the work, because in review, it may get cut back. Don't forget the little things like, where are you going to hang lab coats, put the copiers, etc. Try to involve engineers, architects, etc up front. I have also, in the past began the design of a chemistry lab without this kind of help and it was miserable. It is a huge amount of work but when you are finished it is well worth it.

A5: I would recommend a separate lab for mycological identification but not routine testing. Standard procedures can eliminate cross contamination

A6: If I can just add one recommendation. "clean" and "dirty" operations should be separated, if not by room, certainly by BSC or clean bench. You should only do validation related activities (with know inoculation challenges), streaking of plates, etc. in a dedicated bench (still needs to be cleaned according to a schedule). All product testing, water testing, and what have you should be done in a dedicated bench that is never exposed knowingly. This is a good practice. If you have only one bench, really push for management that this is good risk mitigation strategy. Possibly even supplies that are used for each (dirty vs clean) operation should be dedicated where possible to prevent cross contamination. We'd all hate to wind up with a contamination of product with ATCC 6633. How odd would that be?

Designing a Validation lab

Does anyone have insights related to designing a Validation Lab

A1: Refer to USP chapter 1117 for a general guide line on the flow of Microbiology Lab like division of dirty activity and clean activity.
Lab can be divided into
sample receiving area and storage,
Testing area for non sterile test articles,
Sterility suites if planning to support sterile products manufacturing- classified areas to perform sterility testing (isolators or Clean room).
Documentation area and storage,

Media preparation and autoclave room,
Incubator room or Environmental controlled areas if planning for walk in incubators and refrigerators/ Freezers..
General Storage area for supplies required in micro lab.

Microbial Culture Supplier

I want to know about microbial culture supplier in United Kingdom. Please Help.

A1: Thermo Fisher Scientific has a microbiology division based in Basingstoke offering Oxoid culture media.

A2: maybe
Oxoid Ltd
VP Science and Technology
Wade Road, Basingstoke
HANTS
Great Britain

A3: bioMérieux in UK offers culture media and also the Bioball quantitative/calibrated microbiological strains.

A4: Please call the following culture collection for your needs:

NCIMB Ltd.
Ferguson Building
Craibstone Estate
Bucksburn, Aberdeen AB219YA, Scotland
Telephone Number: 44 (0) 1224711100

A5: both Oxoid and Biomerieux do cultures....

A6: Please see the contact information below:
Oxoid Limited
Basingstoke, UK
Coverage: United Kingdom
Products: Full
Phone: 44 (0) 1256 841144
Fax: 44 (0) 1256 463388
E-Mail: oxoid@oxoid.com

USP Dilution scheme - General notice 6.50.20 solutions and chapter 61 sample preparation

When we make dilution for MLT or other test(s), we always expressed dilution as "1:100" (1 part of sample (solid/liquid) and 99 part of diluent).

As per USP general notices 6.50.20 guideline and chapter 61 sample preparation section, above listed dilution should be expressed as "1 in 10 dilution" not as "1:10 dilution" (1 part of liquid into 10 part of liquid).

I think most of industries are using "1:100 dilution" expression instead of writing "1 in 100 dilution",

Need your feedback

Spiral Plater

I would like to hear from anyone with a spiral plater experience. How easy are these to operate and how useful would be to have one to perform Preservative Efficacy Testing? From the instructions it appears one would cut all the steps in diluting controls and samples and just use one plate per sample - but does it work in practice? Your comments?

A1: We've been using spiral plater to perform disinfection efficacy testing and preservative effectiveness testing. Spiral plater and the reader system are highly efficient and reproducible approach for culture determination of bacterial and fungal viability. The system requires minimal maintenance and gives substantial savings in media and technician time. Preparation of only one dilution tube is required when using spiral plater compared to numerous dilution tubes when using standard plate count method.

A2: Can anyone comment on the use of this system with creams and ointments?

MPN methods, interpretation of results and the "CFU/g thing”

in 5.1.8. chapter of Ph. Eur. 6.7., category A says (among other parameters): Escherichia coli (2.6.31.), acceptance criterion: 10 to the 3rd CFU/g.

If we perform the test acc. to 2.6.31., and prepare several dilutions of our product (e.g. 0.1 g, 0.01 g, 0.001 g, 0.0001 g etc.), which dilutions are allowed to be "positive" if we still want to correspond to the above mentioned E. coli criterion?

To be exact, can the tube carrying 0.001 g of the product be positive? The table included in chapter 2.6.31. says that if 0.001 g tube is positive than we have more than 10 to the 3rd. But what if our fourth tube, 0.0001 g, is negative? Than we have less than 10 to the 4th, right? And that means that we have 10 to the 3rd. Then, if our acceptance criterion, acc. to 5.1.8., is 10 to the 3rd, our product corresponds? Please correct me, if I got this all wrong.

Also, I was wondering about the "CFU/g" thing. The above mentioned criterion (just like in the case of bile-tolerant gram negative bacteria in 2.6.13. and other MPN tests), is expressed through CFU/g. But, how can we express our results as CFU, if we don't have colonies to count? And how does this relate to TAMC and TYMC results interpretation where 10 to the 3rd actually means that our counts can go as far as 2000?

A1: In Ph. Eur supl.6.7, 2.6.12, table 2.6.12-3 there is a table that explain how to interpret you results for the MPN method.

For each dilution you test in triplicate. That's very important for the interpretations of your results! See 2.6.12 for more information.

For example: 2 of the 3 0,1g are positive, 1 of the 3 0,01g is positive and 1 of the 3 0,001g is positive. Check the table (page 5434 supl 6.7) and you will see that the result is 20 MPN/gram of ml product.

It is possible that 3 of the 3 0,1 gram are positive, 0 of the 3 0,01 gram are negative and 2 of the 3 0,001 gram are positive. Check the table and you will see what your result is.

You check the most probable-number per gram or ml product, not the colony forming units per gram or ml. If you want to count CFU's you have to do the plate count method.

You only check the total aerobic microorganism count (TAMC) (bacteria and fungi) with the MPN method! You can't say anything about the yeast and mold. If you want that, you have to do the plate- count method.

You have to report your results in MPN/gram or ml.

suitability of bile tolerant gram negative test method

I would like clarification on the suitability of the test method in the presence of product for the bile tolerant gram negative test.

USP <62> states "use the number of microorganisms equivalent to not more than 100 cfu in the inoculated preparation."

Since the Bile tolerant gram negative bacteria test method does not reference the total volume to use for the test (just states 1 in 10 dilution of not less than 1 g of the product)you may use a 90ml test preparation sample, 99ml test preparation sample, or 190ml, 180ml, etc. Since the volume may change for this test depending on product would you change the inoculum quantity proportionally to the volume used for the test?

fermentation seed

We perform fermentation using e.coli clone to obtain recombinant protein. our batch size is 20 L we perform seeding in two phase i,.e cryovial to seed 1 then to seed 2 and then in fermentation broth. seed 2 is 10% of fermentation broth, and seed1 is 10% of seed 2. incubate each for 10 - 12 hrs. Seed media is LB media and fermentation media is complex media with vitamins.

I just want to know is there a requirement for two seed, cant we just go from seed 1 to fermentation broth to reduce the passage of bacterial cell.

A1: There's no requirement for a two seed approach but you need two seeds to build enough "critical mass" for the larger fermentor. Those in the industry know, after 60 + years of trial and error, that a small seed is not sufficient.

This has to do with lag and log phase growth and what is needed to achieve secondary metabolites. If your process is capable of producing your product from a small seed stage, well then you've been successful where most others have not.

A2: In Cryostate, vegetative cells are in high stress. Giving a passage as is Seed 1 in LB broth helps recover the cells from cryostate to normal vegetative cells. The second passage is given to help cells to be in active log phase, so that when you feed the fermenter with metabolically active cells you get good growth in fermenter. I have seen cycle time increased if you directly feed the fermenter with seed without the 2nd passage.

2nd passage also helps detecting any contamination issues that you may not see in the first passage due to recovery stage.

A3: Single seed or two seed process is product and culture specific. You can reduce one step if you can increase the transfer volume or OD at seed 1 stage Two stage seed is popular where there is a constrain of inoculums or transfer volume. You can plan few experiments at shake flask level.

A4: That makes sense where secondary metabolite production & efficiency are concerned, but for biomass production it doesn't seem to be critical with yeasts in my experience.

A5: Experience at larger scale E.coli recombinant fermentation at the pilot level had a progression of shake - 100L - 1500 L. At the larger scale; shake right to 40,000 L is practiced, and shake to intermediate 4000 L to 40,000 L is also practiced. At the larger scale - fermentor time is important with respect to turnover in terms of downstream integration. This is a function of expression level, cell density, and doubling time.

Personally, I would go from shake to the 20-L to eliminate the additional chance of contamination from phage and extraneous bacteria from that additional transfer. It could also could help to achieve better

expression consistency. You also eliminate one lag phase. Depending on your host, some adjustment in media might be necessary particularly to eliminate chances of protein expression infidelity.

A6: Whether or not you need a two seed process is dependent on your culture. Often, for fermentation processes, synchronous growth of a culture is necessary to achieve optimum process. A two stage process has historically been implemented to achieve this growth.

A7: Two or multiple stage seed or inoculum development is generally done to get the active cells for real process (fermentation). Generally multi step seed development is done to achieve the desired inoculum volume gradually e.g. if you go directly to2 or 5 L from vial (~1 ml) it will find it difficult to grow or will take time to achieve sufficient no of cells in culture, thats why multisteps are preferred. But while going to decide the stages to get the final seed it should be keep in mind that during preparation of cell bank your cells have gone through no of passages and as the no of passages increases cells tends to loose their virulence , stability and productivity. Even in some cases there are limited no of subculturing allowed for same reason. So if possible try to go 1 step seed development.

A8: Most of the answers are true given by all scientists for your question. For Manufacturing of Hepatitis-B type of vaccines the culture used is yeast which is Hansenula polymorpha by some companies. They inoculate one vial of 1.0 ml of yeast culture in 1.0 Litre of shake flask media for 24 hrs. Then transferred to SMALL fermentor of 20 L capacity where it is incubated for further 24 hours. After then transferred to PRODUCTION Fermentor in large scale i.e. up to 100 L media. Here as the culture is recombinant yeast, the plasmid stability test is performed so as to know the intactness of plasmid even after 2 to 3 transfers and number of generations.

Where as in case of Pertussis type of vaccine manufacturing, which is a heat inactivated bacterial vaccine, the culture Bordetella pertussis is not a recombinant type and propagated in the same way as the above method except the small fermentor some manufacturers use second shake flask containing 3 L of media.

There is the difference between two types of manufacturing process where the culture type is different, media used is different, the growth phase is different, early or late growth in media, difference in the way of propagation. Hence study has to be conducted depending upon the type of product.

In short, the use of second flask or small fermentor depends on the type of product to be manufactured.

This is some small information from my side in addition to all other answers.

Spraying water Ports after use with IPA

It is very common practice to spray WFI ports with 70% IPA after use. After reading PDAs newsletter article on dispelling myths. I was wondering if this is something that adds value or performed because it has always been done. IPA is supposed to disinfect the port after use. Many times this same IPA becomes TOC contamination in water samples. The analyst obtaining the water samples sprays the WFI port with IPA and concurrently contaminates the TOC sampling vial. Switching to 6% hydrogen peroxide may be better.
How many people still spray WFI ports with IPA and if not has contamination shown up from not spraying the port?

A1: I assume that you are also spraying the ports with IPA during production use- if not then you are failing to sample as you actually use the system. Flushing is the usual way to remove contaminants before use and should be part of system validation. The use of zero dead leg valves etc. is also part of system design. In a hot system such as a WFI system then sufficient flushing time also involves time at temp to heat up the valve. The key to all this is sample as used and validation. if you have both of these covered you should not have a problem.

A2: I do not think there is reason to spray IPA after sampling for TOC or microbes count.

A3: We have actually had very little issues with disinfecting use ports with 70% IPA after sampling on our High purity water system's TOC values prior to capping the ports. We typically see values less than 100 ppb with 8000 gallons circulating. I am just becoming familiar with WFI, so I cannot speak to TOC issues there, but with valves closed after use, and a sufficiently large enough volume of water in circulation, I am not certain that a little bit of alcohol can impact your numbers.

Uncommon recovery with tween 80 neutralizer

i need to know if tween 80 affects in some degree the growth of P. aeruginosa because during Validation of microbial recovery of syrup the count of challenge inoculum control in buffered solution with neutralization method is below than neutralized product with inoculum, nevertheless the count between inoculum in absence of product or neutralizer (viability group) and neutralized product with inoculum are comparable

Neutralizer: tween 80 5%

(1) neutralized product with inoculum: 66 cfu

(2) challenge inoculum control in buffered solution with neutralization
method : 37

(3) viability group: 83 cfu

USP says that "can be established by directly comparing the result in the treated solution (1) to the inoculum (3) above

I know that It is possible that polysorbate alters the permeability of the cells, since it has been found that polysorbate 80-treated bacteria leak intracellular constituents and become susceptible to changes in pH, temperature or sodium chloride (NaCl) concentration

Second case

For the C. albicans in SDA, groups 1 and 2 obtains a greater count to group 1. In this case tween 80 would increase the count

Sampling compress gas and nitrogen

Anyone knows any requirements on the frequency, tests, limits for sampling compress gas and nitrogen (viable or non-viable sampling).

A1: For viable sampling we sample both of them once a month from all points (generator & sampling points).

About the limits: Less than one (nil) in the sterile plants. The problem in sampling devices. Check suppliers in your area.

A2: Check out ISO-8573, it all depends really on how often and what you are using the gasses for.

Sterility test invalidity

The following theoretical issue was raised at our site:

Performing sterility test according to USP <71> or Ph.Eur. <2.6.1.> appropriate negative controls should be included. The pharmacopoeial text is clear concerning the negative controls when microbial growth is found in the product sample. Here, the text says that the test may be considered invalid only if for

example microbial growth is found in the negative controls (and of course the contaminants are identical based on genotyping).

But what about the situation when there is no growth in the product samples, but there is growth in the negative controls. Should we also consider this test invalid based on that the test was not performed properly and there was something wrong that cannot confirm the negative result, either. Or we can accept that the product meets requirements for sterility independently from the control results.

I ask this because I have found references for both approaches (and I can feel the logic behind both).

A1: The validity of a sterility test result is based on certain acceptance criteria, which include "No Growth in the Negative Control". I would therefore invalidate the results, conduct an investigation of the negative control failure, determine the root cause, correct the problem to prevent recurrence, and repeat the test.

A2: This is one of those situations where you need a microbiologist and not a chemist or a lawyer. The function of a sterility test is to confirm sterility not to assure it. Sterility is legally defined by a number of factors including "passing" the sterility test. In the case you are describing one of the first things that came to mind is that your product was inhibiting the growth of whatever was coming up in your negative control. This could bring into question all your sterility tests in that something is in the environment that could contaminate your product but could not be detected by your test.

A3: Technically I'd see the test as invalid.

A4: The contaminant could be inhibited by the product during the test.
I suggest you, to perform a recovery test with that microorganism (less than 100 CFU).

A5: We observe result with reference to negative control if negative control showing growth it is positive, so test requirement does not comply. If negative control shows growth means something wrong with analytical procedure, how we can proof that our test procedure is right. If there is any justification please share with me.That topic is most interest of discussion.

A6: Your sterility test obviously is canceled due to growth in negative control. We often use the term as " Investigate the root cause", actually it is very difficult to find out the root cause or the source of contamination for sterile product. During sterility test negative control may be contaminated and it is not only depended on media sterilization, also depend on analyst performance on aseptic technique. Your product showed negative in test, it does not mean that your culture media were not perfect for growth promotion test. Contamination may be occurred during negative control testing.
Negative control included as below:

a. Solvent which you use for dilution of product that means rinsing or neutralizing solution
b. b. Any other device used in test such as disposable syringe for dry vial reconstitution
c. c. All apparatus used during test in absence of product.

Last of all my opinion that you must be repeated this test and that test will be as first test. That is the sterility test regulations.

A7: Thank you for your comments (I copied some of them below). I can accept your reasoning concerning that the positive negative control refers to something wrong in the procedure. My only concern is that the repetition of the test could mean additional risk to the product itself in some cases. For example this is the case when sterile bulk is tested, and the re-test necessitates additional sampling from the bulk. This is why I wouldn't like to implement an automatic cancel for the test. Yes, you are right, it is very difficult to find an unambiguous root cause that could not have any effect on the product testing, but what about the situations:

when after the incubation period, a till then undetected leak could be observed only on the concerned negative control canister?

or when clear evidence can be obtained that the contamination in the negative control is the same that could be cultured from the operator's fingerprint? Yes, this refers to poor aseptic technique, but if a method suitability test would confirm that the contaminant can be detected by our method? It would mean for me that the negative control was unfortunately contaminated but the product test remained inviolate.

What [name redacted] n wrote is another thing. In that case, the method suitability test with this new isolate would fail, and this would really query our procedure.

So, based on the above, I tend to perform a a deep investigation, and if an obvious root cause could be identified that could not have any effect on the product result, in that case I would accept that the product meets the test requirements.

Or is it dangerous?

A8: The test is invalid and the examples offered are merely endorsing that fact. You can't pick and chose the result that suits you. The finger bug example merely proves the operator was (at least part of) the problem. So why do you accept any results from this test by the operator who was shown to be unable to correctly perform the test.

Too often the focus of discussion is release of the product. Please consider the larger issue - if your scenario is the root cause, all tests by that operator may now be in question. Have you retrained that person?

A9: Would you repeat the test if you do not find "an obvious root cause"?

A10: Hi Peter,

My interpretation, and I believe, the one that regulators usually apply is that in the case of a Negative Control failure only, the batch is able to be released, based on the assumption that the contamination occurred in the sterility testing suite, and is not related to production activities. There is also an implicit assumption required that the negative control was taken from a previously declared sterile batch.

Of course, this standpoint is much better supported if you can find a smoking gun of the same species of bug in an environmental sample from the sterility suite at the time of testing.

[name redacted] and I agree to disagree on this, and I'm sure he will have a strong opinion.

A11: All, In addition to my previous post where I suggested that you can release this batch (flying in the face of popular opinion apparently!) I also note with some concern that two respondents suggested that there could be a difference between the product under test, and the negative control such that the product could be suppressing growth that is manifesting in the neg control...! There had better not be! What sort of a negative control do you think you are using if there is even a hint of a possibility that it could be that significantly different from the product under test?

The whole point of a negative control is that it is IDENTICAL to the product under test in order that this argument CANNOT be used.

BTW, I stand by my RELEASE decision: The contamination is restricted to the sterility testing suite, and if you've done the procedure correctly, and the neg control was performed last, then the evidence points to an operator getting tired and sloppy, but it does not in any way suggest that the production environment was contaminated in the same way. To assume that a negative control testing positive is indicative of a production failure is not supported. In fact, this scenario provides some evidence that a contaminated Finished Product batch would have actually been confirmed as failing by this test method, using the same logic as applied to Stasis testing.

A11: The fact that a negative control was not, in fact, negative should not invalidate a sterility test if the test itself was negative for growth. UNLESS, the product used in the actual test could not support the growth or survival of the contaminant found in the negative control. The negative control is intended to show that there is not some intrinsic contaminant in the test system including the environment that could contaminate

the actual test material during the testing procedure thus bringing into question whether any contaminants were, in fact, in the actual product. However, in this case the actual test was negative. So why would you want to negate/invalidate a negative test when a control was found to be contaminated and not the actual product tested?

A12: Sterile is Sterile. Just because some other part of the test was performed incorrectly does not change the STERILE result for the product. To return a STERILE result means that the samples tested had to be STERILE.
The fact that the neg control was positive ONLY shows that the operator stuffed up the Neg Control part of the test, (but they obviously got the Product part correct...anything other than correct is a contaminated test sample, which is not the case). Furthermore the presence of a contaminated neg control shows that if the product samples had been anything other than STERILE, then the test would have identified them ergo, Test negative, control positive is a releasing scenario.

Yes the operator needs retraining, but it does not call into question any previous results. It is a simple logic thread; for any test to return a STERILE result, the samples had to be sterile in the first place. QED.

A13: I have to disagree with [name redacted]; I agree with [name redacted].

The negative control in a sterility test could be useful when both it and a product test turn out positive. The product test itself should have been validated by the standard bacteriostasis/fungistasis test. The media should have been purchased, or prepared in a validated autoclave, and been subjected to both a growth promotion test and a
sterility test (the sterility test preferably testing the entire batch before use). The technicians should have received suitable training. In short, if GMP is followed here then one should be able to rely on the negative results of the tested product(s). The negative control failure really has no bearing on them.

So why a negative result failure? One possibility, touched on by [name redacted]: if the negative control is done towards the end of the sterility test session, and if the company concerned has a low incident of sterility test failures (which it should!), I wonder if some technicians subconsciously become more sloppy whilst performing the negative control. Whilst this may not be commendable, it could explain the failure. It does not necessarily reflect any failure of the technician to perform the product tests correctly.

I would release the products.

A14: Then why run a negative control?

You imagine the most favorable scenario for the results generated incorrectly by the technician, by mistake or intent. Imagine a less favorable scenario - the samples were mislabeled.

A15: Some would like to look at the value of a negative control from a perspective other than just providing an "alibi" for the product. Controls are there to validate the integrity of the test procedure that includes the quality of reagents, components and performance of the analyst. A negative control that shows growth is an indication that the test procedure is compromised. If a defective component or reagent that is specific for the control is shown to be the culprit then the problem is solved. But if as many suggested the isolated growth in a negative control is an indication of an analyst error, incompetence or oversight, what makes you so sure that the analyst messed up only on the negative control? How can one be sure that the analyst did not mess up with the tests samples in a way that does not favor growth if there was a contamination? like test inadequate sample, not enough neutralizer etc. If you are prepared to blame the analyst's incompetence or error (since most agree that the analyst needs retraining), it is kind of hard to assume that his/her transgressions are limited to the negative controls only.

A16: The product was validated using only strains that are described in USP; but you don't know if the contaminant can be recovered following the employed method.

1) If you demonstrate that the contaminant can not be recovered in the product, but it can be recovered in the negative control (without product), you can imagine that maybe, using an adequate method, both, the sample and the negative control could be positive. And if both, sample and control tests are positive, the decision of invalidate the test is the correct one, according to USP <71>.

2) But if the contaminant can be recovered in the sample test through the employed method, you can be sure that the contaminant never was in contact with the sample test. So the decision of release the product (without retest) is correct.

If 1) or 2) are the scenario, the cause of the growth has to be investigated.

My suggestion is to perform the bacteriostasis test with less than 100 CFU of the contaminant, before to take a decision.

A17: Obviously one possible chance is touch by technician during Negative Control test. Yes, it is wise performance to run negative control test at the end of product test. At last session I talked about this contamination. As negative control failure, no one can decide the product is passed, it will be under investigation. If anyone release the product, it is not wise decision and it is avoiding tendency. So negative control failure is not negligible matter.

A18: I found this discussion very interesting and thought I'd add my two cents' worth.

It is true that the organisms recommended for BF testing can be seen as few. However, these organisms are meant to act as representatives of the various groups of microorganisms that would more likely be present in the manufacturing environment. Casting a doubt on the ability of these organisms to adequately qualify the sterility test method invalidates BF testing altogether. That is, we might as well qualify this method using every species and every strain of microorganisms available, which is nearly not possible.

I think that a positive negative control should not invalidate the results of the negative test samples.

I understand the rationale that if the negative control was contaminated, it could mean that the test samples may have been contaminated - but the contamination failed to show up because the product was unable to support the growth of the contaminant. However, in the same token, the rationale could be applied to the cases when all the samples (both the negative control and test samples) were negative. In this case, are we going to think that the test samples may have been contaminated one time or the other, but the product failed to support the growth of the contaminating organisms? This would mean that whether or not the negative control fails or passes, the test samples themselves could be invalid because they may have been unable to promote the growth of any contaminating organisms.

If the negative control shows growth and the product itself may also have been contaminated but failed to support the growth of the contaminant - doesn't this show that it would be highly unlikely that the contaminant came from the product in the first place?

A19: The negative control is used for two reasons. The first is to show that the analyst was able to perform the test without contaminating the samples. The second is to link a growth in the sample to analyst error. If there is a growth in the neg and a sample you can draw stronger conclusions to analyst error. Having said that, re-testing is not taken lightly and there are larger test samples that will need to be performed even if the you end up linking the negative control to the positive sample. (Check you compendia for the acceptable responses to a sterility failure).

[name redacted] was correct, a positive result in a negative control does not change the fact that all of the samples were sterile - after all that is truly what you want to know.

You need to do a full investigation with a write-up and re-train the analyst if there is no error/issue determined or analyst error is determined. Most places I've been use an UnExpected Result (UER) system to investigate these kinds of results/issues.

A20: You present a compelling case, but I fear that the question is actually far simpler.

1 - the Sterility test is manifestly unsuited to meet the implied function in its title; it cannot test product sterility.

2 - The Sterility Test exists to meet a monograph obligation to "meet the requirements of the test for sterility" That we choose to apply the test universally is a decision that carries consequences, one of which is that we have to adhere to the conditions set forth in the test.

3 - The test is therefore a requirement, not a well-designed assay of finished product quality

4 - Growth in the negative control may serve to invalidate the test as per the language of the test.

At a certain level we are at a basic GMP requirement, and at a strong desire to avoid the appearance of impropriety. If we only observe our controls when convenient, what is that saying about our operation?

A21: Well, finally!
You've been very quiet on this, a subject I know you have strong opinions on, [name redacted]; welcome to the discussion!

And, as usual, you provide succinct and erudite comment, to which I cannot resist responding to.

Sure there are issues with the test, but as we all recognise, it's a necessary evil thrust upon us by the regulatory interpretation of the monographs (unless we're fortunate/good enough to be granted parametric release).

The Conditions set forth do NOT insist that a standalone negative control fails the test; Read the "Observation and Interpretation of Results clause carefully! (BP 2009 Appendix XVI A401-A402) It clearly says that IF the Product test shows growth, THEN, one or more of the following (a-d incl.) clauses might be used to invalidate that result and perhaps subsequently release an apparently failing batch. There is NO direction regarding the scenario we have been discussing exactly because it's a no brainer; NO Growth in the product is a PASS and we do not need to apply or consider any of the clauses (a-d).
I'll say it again; the clauses a-d are only to be applied in the event the Product shows Growth. Yes, this does mean we can "ignore" a negative control if the test passes, although as we all agree, to do so would be foolish and we will of course perform a full investigation and rectify the cause of the contamination, but we will not be required or expected to have rejected the batch.

If in this scenario we take the logical option to override a growing negative control, we are not necessarily "ignoring" it (and our investigation will support this), but we are applying our knowledge, experience and correct interpretation of the compendial test to release an uncompromised batch to market. We are industrial microbiologists and our brief is to support our employing business in making a profit as well as a safe product. I cannot in good conscience throw out a $500K batch of product just because my techie got tired and stuffed up a negative control; indeed my boss is savvy enough to fire me if I were to suggest it.

It does occur to me that perhaps many of my most vocal opponents on this issue might not be aware of my assumption that Sterility Testing is performed in a dedicated part of the microbiology laboratory at a great physical remove from the production environment. If, of course the testing is performed in the same LFC as product filling originally occurred then I would agree that the scenario is cause for complete and immediate failure of the batch, based on: the filling environment is compromised and a real risk exists that one or more units of product could have been compromised by the same contamination event that the control picked up.

How many more ways can we bounce this around?

A22: For me, this is really simple - if you run a control you adhere to the results of the test. This discussion is repeated every view years, and every time it sounds to me like we want to use the negative control only as a "get out of jail free" card.

If you run the control, you consistently interpret the results.

A23: So you'll document that in your SOP's - growth in negative controls in absence of observations helpful in the investigation will not invalidate otherwise appropriate test.

A24: I think the differences of opinion we are seeing is because there is not guidance in the compendia for a positive result in a negative control. So as a company, you need to decide what your response should be in this case. Perform a full investigation including identifying the organism in the control. (You could go as far as inoculating a 10-100cfu/ml suspension into the test samples to show that this organism will grow in the test samples. Though this opens a big can of worms if something goes wrong - including putting the validity of your sterility validation/B&F into question)
As part of the corrective action for this, you will need to use your CR system to add into the SOPs what a valid test is, including how all of the possible combination of test results should be interpreted. This will create consistent interpretations of results and what will need to be done if the results are undesired. In this case, the SOP could say that no investigation is necessary, or that a full investigation is required and the results are invalid, or something in-between. But the investigation that you do for this test should be guidance for how you move forward with updating your SOPs.

A25: I agree that sometimes the compendia may be grey in its interpretation of microbiological testing results. That is when one has to go beyond the compendia and consult other guidances, and with regulatory and industry experts for help with an interpretation that will most likely be acceptable to the regulatory agencies. In the chapter on Sterility Test in the book "Parenteral Quality Control" by Michael J. Akers, et. al (2003), and the section on "Control in Sterility Testing" p. 52-53 there is an interpretation of negative control positive. In that section, the three renowned industry experts and authorities in pharmaceutical microbiology state that "If microbial growth is detected with a negative control, the medium was not sterilized properly, contamination was introduced accidentally during the test procedure, or there exists an inefficiency in the container or packaging system. If the microbial growth in a negative control occurs and in the absence of evidence from the environmental monitor, equipment, or personnel of accidental contamination, it becomes a clear indication for retesting the product."

While the above opinion by these authorities may have more logic and compliance than scientific component, unfortunately, it is likely to be the thinking of most regulatory agencies., rather than considering only the product test results. Yes, an investigation must be performed to identify the root cause, if possible, but it will be very difficult to come up with any regulatory acceptable conclusion that will allow only the product test results to be used to pass the test without considering the negative control positive.

A26: Hi [name redacted],
For once I must take you to task in regard to the content of your post; we are indeed "interpreting the result"; it's just that we are discussing/debating the appropriate way to do so consistently.
And note that the compendial reference itself offers the "get out of jail free" interpretation, not just me...

I firmly believe that my standpoint is the correct one. I submit that we as scientists have a duty to explore, challenge and question everything all the time, and not just take the "safe" option. In this instance there are several implications and upshots of interpreting a batch failure from just the negative control contamination.

We could question the sterility of the negative control itself; thus casting into doubt the safety of the batch that the neg control was sourced from. (understanding as we do, the shortcomings of the sampling regime from which we source the sterility samples, this might not actually be quite so farfetched as it sounds?)

We could accept that the negative control was accidentally contaminated after the test samples had been correctly handled.

We could take the situation as suggested by many of my detractors, and impugn the quality of the test batch, based on the failure of a completely unrelated unit, incurring the wrath of production, and destroying the credibility of QC.

When reviewing the possible results and the scenarios that might cause them, in specific regard to the Sterility Test, we must start with first principles; What is the Failure Mode of the test? Answer; Growth in the vial(s). If there is NO Growth, then the samples MUST have been Sterile. This is simple, incontrovertible logic. Furthermore, if a previously declared "Sterile" sample used as a negative control, returns a Positive result, then that contamination must have occurred in the testing environment and is de facto an artifact of the test procedure/environment, NOT a reflection of the quality of the test batch, or the filling environment in which it was manufactured.

Remember, for a unit to pass sterility testing, it has to be a) sterile and b) handled correctly during the testing procedure. In this case, for the tested batch, both of these conditions must have been met, otherwise the result COULD NOT have been No Growth. So, note condition a)! For the negative control, condition a) was already met (otherwise we couldn't have chosen it as the negative control), and so condition b) must be the cause of contamination. In that case, the problem clearly lies just with the way the neg control was handled and is specific to the sterility testing suite, it has nothing to do with the way the test batch was manufactured.

"Get Out of Jail Free"? NO; ACQUITTED; never was going to jail, no case to answer.

And Yes, [name redacted], if we are so unsure of our ability to apply logical argument to a regulator, then of course we can document this interpretation in our SOPs. I would, on the other hand, simply refer them to the compendial clause I cited earlier, and ask them to point out to me, where it says that I have to reject a sterile batch just because a neg control was stuffed up...they won't be able to.

A27: When did an "Invalid" test became a "Failed" test??

A28: A positive negative control only tells you something about the analyst or room the test was performed in. The negative control does not have product in the sample so tells you nothing about the sample. The analyst needs to be trained over.
Just think what would happen if the analyst selectively contaminated a product sample.
It could be a million dollar mistake.

It would be crazy to say a negative control turning positive fails a negative product result.

A29: The sterility test is really a misnomer in that it has only a limited capability to detect contaminants within a specified range of parameters. There are any number of organisms that can, in fact, be in a product or sample and will not be detected by the "sterility" test. To assume, and that is what you would be doing, that a sample that passed the sterility test is, in fact, sterile.

A30:
You are proposing one of the biggest mistakes possible in investigating a microbiological failure (which is what a positive negative control is). That failure is to assume the cause without investigating it or having a basis in scientific fact for the cause. You are unwilling to consider specific possible causes for political not scientific reasons. As a test for "sterility", the sterility test is very poor. This is based on statistics and science concerning its lack of ability to detect all contaminants at any level. Assuming you have an aseptically filled product with a 10-4 SAL that "passed" an initial sterility test and you are now using samples of that lot for negative controls in another sterility test why would you consider it impossible that there could actually be non-sterile parts of that batch that you used as a negative control.There have been and will be recalls for products that passed a sterility test and were subsequently found to be non-sterile. The definition used for sterile products is a legal statistics based definition not a scientific definition.

Consequently, sterility is not based on absence of life (science) but probability of a contaminated unit not absence of one (legal/statistical).

A31: I do agree that "growth in negative control/no-growth in sample" scenario is not having sufficient scientific justification to re-run the sterility test. I also agree that sterility test is a flawed test and passing sterility test alone is not a proof of batch/lot sterility. Sterility test is a compendial requirement, and invalidating the test when growth observed in negative control seems to be a compendial requirement. USP FAQ on Chapter 61 says, "If a positive result is obtained with a negative control, the test can be regarded as invalid and may be repeated." http://www.usp.org/USPNF/pharmacopeialHarmonization/genChapter61FAQ.html#Q4

Negative control in microbiological testing is somewhat similar to 'system suitability' in chemistry tests (forgive me) such as HPLC. One of the problems is that microbiologists have to wait for system suitability results for 14 days and by that time sample testing also completed.

If negative control is run at the end of sterility test (e.g., after 5 samples) there is always a chance that operator may be blamed for failing negative control. Running negative control at the start and end of the test may be an option (like bracketing standards); although I am not willing to adopt this approach in my lab.

Consistent interpretation of negative control is much easier to explain to regulatory auditors. Science triumphs regulation; unfortunately in some cases it takes lot of time to do that. Until then invalidating the sterility test when there is a growth in negative control is a best option.

Steam Sterilization of Liquids

When validating autoclave sterilization loads for liquids, does the microbiological data from biological indicators supersede the temperature data? I performed a load validation for 1L bottles of Dilution Fluid A that did not come up to 121°C during a 45 minute cycle; the "come-up" time was approx. 15 minutes and the dwell temp. oscillated around 119°C. All BIs in all three loads exhibited no grow upon incubation. Should I investigate the performance of my autoclave (calibrated within the last 8 months) or thermocouples (calibrated immediately before use)? Or is this considered an acceptable validation? (BI D value is 1.5 and spore count is 105)

Also, do different liquids exhibit differing challenges to steam sterilization (i.e. distilled water as opposed to liquid media)?

A1: The question I have is the "dwell temp.". Are you utilizing a load probe or are you taking about the probe (RTD) in the drain? If the RTD is in the drain, then something may be wrong with your control system or the sterilizer is at or beyond capacity in terms of total sterilization volume. The controller should not start exposure until the temperature of the sterilization environment achieves the set-point temperature (e.g. 121.1 Deg. C). If it falls below the set-point, the controller should not accumulate exposure time until the set-point is re-achieved. If the dwell temperature is achieving set-point and then continually going below the set-point then you may be experiencing excessive condensate build up and the sterilizer is adding make-up steam because the load is at or beyond the capacity of the autoclave. Another issue may be poor steam quality (continual make-ups).

Are you validating your sterilization challenge via overkill? If you are using the overkill method, then for a 45-minute intended exposure period, then you would challenge a 22.5-minute cycle (half cycle).

As for liquids, they generally have a density equal to or slightly greater than water therefore a water challenge is applicable unless you are intending to sterilize something with a significantly higher density in comparison to a water phantom (e.g. 4X media, concentrated solutions, etc.). If you are concerned with concentrated solutions a quick penetration study can be employed to document the relative challenge (Water Vs. Conc. Solution). Once the penetration study is complete, one would select the greatest sterilization challenge and employ said challenge in the validation.

A2: The first thing I would consider is the calibrated range of your thermocouples. The last GMP lab I worked calibrated them to +/- 2 degrees. If that were the case, then 119=121 and would be acceptable.

A3: This is not a satisfactory performance for the autoclave. Is there air in the chamber?

A4: The come-up time requirement is for dry goods loads. Liquids loads will always lag behind the chamber. You can use product Fsub0 to estimate the equivalent time at 121C. I would say your spore count is a bit low, though. A D value of 1.5 and a spore count of 10^5 does not give you an FsubBio of 12 or greater (FsubBio is derived from the spore log reduction and D value of the indicator organism). PDA Technical Report No. 1 - the revised edition - gives a very thorough explanation of the concept.

I suspect the actual D value of the biological indicator is higher than the manufacturer's rating. Are you using an indicator ampul? Most manufacturers give the D value of the spore suspension INSIDE the ampule. That is, they place the suspension on a filter paper strip for D value testing. The thermal lag introduced by placing the suspension in an ampul can be significant.

As far as your 2nd question, the answer is yes. Different liquids can have vastly different challenges to steam sterilization. Distilled water is not equivalent to liquid media. If you want to use distilled water as a surrogate for various media, you will have to perform a study in which you inoculate DW and the various media for which you want to substitute DW, and expose them all to a marginal sterilization cycle. If you can demonstrate that the DW repeatably grows out when the inoculated media do not, you can use the DW as a surrogate for the media. Obviously, the study I describe is not a trivial task, and will require a significant amount of thought and planning, such as recovery of the endospores from the inoculated liquids, incubation space, etc., etc.

A5: The physical and biological acceptance criteria are treated equally by regulatory agencies. However, the physical criteria is based upon the cycle parameters. The liquid does not necessarily need to reach 121.1C. It does need to have an accumulated lethality (Fo) of greater than 12 minutes to meet the requirements of overkill sterilization with an SAL of 10^-6.

Large volume liquids have a longer come up time than the chamber and are generally controlled with a load probe located within a container.

Water heats more quickly than thick media. If you graph the thermal measurements you should seed that the liquid is gradually coming up to temperature.

With heat labile liquids it is important to minimize the total accumulated lethality to reduce degradation. There are alternative to overkill sterilization strategies for heat labile liquids.

A6: Additional insights:

--Plastic bottles are slower to heat than glass or steel.
--Overkill sterilization methods require a minimum of 1 x 10^6 spore population. Are you using overkill or bioburden sterilization strategy?
-- If your acceptance criteria requires the liquid reach 121°C then it is not an acceptable validation. We would need to know more about your acceptance criteria to determine.
-- What is the dwell temp. setpoint and is a load control probe being used? -- Is the dwell period based upon Fo or time

A7: You should meet all criteria for the temperature and BI/Ampoule kill.
(Just to confirm, you are using BI ampoules dropped into the liquid or BI strips dropped into the growth media (SCDM or TSB), and not BI strips suspended in the chamber.)
If you did not meet the temperature, and you choose to use this run as a validated run, you need to perform an investigation and write up a deviation to the protocol explaining what happened, and why you

are willing to accept the results as the final validation. You will likely need sign off from your validation management and QC/QA management.

Different volumes of liquid will change the length of time it takes to get up to temp/pressure. The kind of media should not affect the run noticeably.

Another issue that you might want to look into is how "dry" your steam is for your autoclave. Steam that is closer in temperature to the boiling point of water will not heat up your load as quickly or as high as steam that is higher in temperature/dryer. There is a way to determine the dryness of your steam but I am not familiar with the name of the test. Our Facilities group tests this on an annual basis.

A8: Check the standards used for calibration of TC's and the RTD probe of on the autoclave. if both of them are calibrated using different standards check the protocol regarding your acceptable temp difference/sensitivity, generally its +/- 0.5C. Also review your Cycle development runs for the 1L bottles of Dilution Fluid A at that time did the temperatures scored by TC's were up to 121C, if they met during CD and the load configuration does not change with position of TC's there might be problem with TC's calibration or RTD calibration on Autoclave.
Theoretically both physical and biological acceptance criteria are predefined in the validation protocol so if in these instance temperature is not meeting the acceptance criteria, I would suggest tweaking the cycle parameters and repeat CD, verify by TDHP and upon satisfactory results commence BI challenge.

To your second question, yes the heat penetration would differ upon the medium constitution, some takes more time to heat up and others less. Generally viscous material takes more time then like Agar media takes more time compared to broths. so determine the worst case and bracket your loads accordingly for BI runs.

A9: Just as an FYI, something similar recently came up where an FDA inspector refused to allow just a deviation and accept the documented rationale why the failure was acceptable. They expect this investigation (or should say this inspector expected) that you have the justification and you fail the run and would perform the run again.

A10: You have the most responses to a question that I've seen in quite awhile. You'll have lots of stuff to consider. I agree with most all the help offered.... I may be a bit biased but regardless of what my instruments tell me, if I can't kill the BI, I'm very much concerned and as most have commented, start an investigation. But, as in your case, BI dead but TC's said only 119C. I really do not see a problem here as long as you have met your acceptance criteria.... Fo of 12 and 100% of BI lethality? If the criteria is 121C for X number of minutes, then relook at criteria. For your liquid loads, long come-up can easily meet the Fo criteria but tough (and needless) to actually hit the 121C IN THIS CASE. In other situations, yes, the 121C may be necessary. We have some large volume liquid loads that as with yours, hit a high of 118 or 119C but Fo is around 25+ with come down. Our acceptance criteria of BI lethality and Fo of 15 minute minimum is hit and load passes. This is a good justification for stating chamber temp set at 121C, Log 6 BI lethality and F0 of minimum of 12, 15, or what you decide upon. Now you have chamber temp , lethality and minimum Fo. Three criteria to hit.

A11: Yes, I was pleased to see this as such a "hot" topic.

I was inclined to justify the successful sterilization load based upon microbiological (BI kill) results alone. Admittedly, Fo was not written into the acceptance criteria, as it should have been.

The BI kill results tell me the load is sterile, but I have no idea to what SAL it is sterile, but seeing as how DFA is not a parenteral or drug product, I am inclined to believe that a calculated equivalence to 15 min. at 121C and total kill of BIs is a perfectly acceptable acceptance criteria. One more question though, if I am not establishing a 12-log reduction (6-log reduction of 6-log spore count), only an Fo of 15 minutes, what BI spore concentration and D-value should I use in challenging the load? Do I need to use worst-case D-value of 2.5 and spore count of 10^6?

Also, seeing that BI kill is occurring during such long come-up times, has anyone calculated the accumulated lethality over kinetic temperature as a function of time? That is, the BI was exposed to, say 3 minutes at 115C, 4.5 min. at 116C, 6 min. at 117C, etc. etc.????

PET for w/o products

I would like to ask- does anyone significantly change their Preservation Efficacy Test protocol when you deal with products that are formulated water in oil?
if so, what are the key differences?

A1: Preservative test should reflect the contamination events anticipated for the product.

Contract Lab for Rabbit Pyrogen Test

Anyone know of a contract lab that can perform the rabbit pyrogen test?

A1: CHOKSI LABORATORIES LIMITED
6/3.MANORAMA GANJ
INDORE,
M.P. - India
Phone: +91731-2490592, +91731-2493592
E mail - indore@choksilab.com
Website - www.choksilab.com

A2: There is a lab in Irvine California Called "Biological Test Center". they used to perform the rabbit pyrogen test. I am not sure if they are still doing it but you can go onto the website to find out.

A3: Charles River

A4: A facility in Bangalore, India by name Bioneeds has the capacity and approval from Drug Controller, Goernment of India for conducting Rabbit Pyrogen Testing. The facility is utilised by industry for testing.

A5: There are three in Europe that I am aware of.

They are:

Charles River Preclinical Services Ireland (CRPSI).
Carrentrila
Ballina
Co. Mayo
Ireland
Tel: 00353-96-70355

Charles River Endosafe France
E.S.D. Romans
01400
Chātillon sur Chalaronne
France

Scottish National Blood Transfusion Service
Castelshaw Building
Pentlands Science Park
Bush Loam
Peniciuk
EH26 OPZ

A6: Ethoxint in Rush, NY can. Donna's Ventura would be your contact there.

A7: Pacific Biolabs used to do it. Not sure if they still do.

http://www.pacificbiolabs.com/

551 Linus Pauling Drive

Hercules, CA 94547

Phone: 510.964.9000

Fax: 510. 964.0551

Email: infoweb@PacificBiolabs.com

A8: NAMSA

A9: Check with Wuxi Apptec in St Paul.

A10: You can contact the following contract lab. in Scarborough, Ontario:

Nucro-Technics
2000 Ellesmere Road, Unit 16,
Scarborough, Ontario M1H 2W4
CANADA

Tel: 416-438-6727
Fax: 416-438-3463

A11: This testing is performed by WuXi AppTec, Inc. - St. Paul. Contact Jean.Mesarich@wuxiapptec.com or go to the website www.wuxiapptec.com.

A12: Wickham Laboratories do in Hampshire UK. www.wickhamlabs.co.uk

Nitrogen line validation

I wonder if anyone can help - I have an aseptic manufacturing process performed in a grade A environment (isolator) that requires nitrogen sparging of the vials during the process. However I am having difficulty in validating the nitrogen 'filling' process as I keep getting particulate alarms going off.

To set the scene - we are trying to see what particulate counts we get from the nitrogen line inside the isolator, we have a filter on the gas line prior to going into the isolator, a filter on the line as it enters the isolator, then a final third filter directly on the end of the line that we are using as a filling tip. The filters are all gas filters and of a pore size 0.2um. We have a diffuser which attaches to the particle counter that diffuses the pressure from the line and allows particles to be counted - however in order for the diffuser to work the pressure needs to be around 3 bars and the pressure during the filling process is around 0.8 bars, so this is also giving us a problem. Even with the three filters we are still getting particulate alarms ranging from 0.5um to >10um. (The air line into the room has been tested and we did not get any particulates, so the supply is fine.)

Any ideas anyone?

A1: This is really surprising to have high particulate counts even after three filters. Have you checked for the moisture contents.Secondly there could be a leakage at some point of connection in the diffuser and particle counter.

A2: The problem sounds very much due to faulty sampling.
Please check the diffuser. There is no way you should see particles after 0.2um filters if all of them are not compromised.
The high count could be due to several reasons. I had seen this type of high count for our N2 system supplying the Lyophilizer.
1) The red cap (may vary depending on the type of diffuser) of the diffuser must be completely removed while sampling.
2) There is a O ring inside the diffuser that needs to be changed or needs to be cleaned periodically. Talk to the vendor, they will be able to provide some information too.
3) Not sure, what type of particle counter you are using, but some counters pull air from the environment while trying to maintain the airflow.

In our case that was exactly happening. After removing the diffuser and controlling the air flow from the main source along with the particle counter the results were always 0.

Could we use reagent grade materials?

I've a query about the use of reagent grade materials for manufacturing Non sterile/Ophthalmic/sterile dosages forms?

Does anybody give me any suggestion ?

In USP (General Notices it is as- Should comply with the USP Monograph) but if a reagent grade material complies with the USP specification, could it be used for manufacturing pharma product? As we know Reagent grade are highly purified/but not for Human consumes ion.

A1: Reagents described as such in the pharmacopoeias are suitable for use as reagents. It cannot be assumed that they are suitable for use as ingredients in products.

Other reagent grade materials may be suitable for use in pharmaceuticals if there is no pharmacopoeial monograph. This would need to be included in the marketing authorisation application and would need to be approved by the relevant agency.

Where there is a pharmacopoeial grade of material available it should be used. Whether testing to the pharmacopoeial monograph would be adequate to assure that a non-pharmacopoeial grade of material is suitable for use will depend on how the substance was manufactured, its impurity profile, etc.

A2: If a USP/NF grade is available I strongly urge you to use it as it is a regulatory expectation

conductivity question

I know that this is not a microbiology question. Please forgive what is, ostensibly, a chemistry question. Some years ago, I had an article that described the specification setting rationales for the current USP Conductivity requirements. It was a great article, but now I cannot find it. Does anyone have either the article, or the reference? I would love to get another copy.

A1; This article is cited in Collentro's text, "Pharmaceutical water: system design, operation, and validation" as a discussion of the conductance effect of each ion in determining the specification. Hope that helps.

http://us.mt.com/global/en/home/supportive_content/know_how/Paper-THOR-Cond-Pharma-Bevilacqua-01-01.rxHgAwXLuMvM.MediaFileComponent.html/cond_tests_pharm.pdf

Enterobacteria and MPN

USP and Ph. Eur. both deal with samples of natural origin (for example teas).

USP 31, page 721, includes a table for the MPN of Enterobacteria that says:
if 0.1 g tube is -
if 0.01 g tube is -
if 0.001 g tube is -
than MPN of Enterobacteria per g is fewer than 1.

Ph. Eur. 6.7, page 5446, also includes a table for the MPN of Enterobacteria and says:
if 0.1 g tube is -
if 0.01 g tube is -
if 0.001 g tube is -
if 0.0001 g tube is -
than MPN is <10.

USP seems to be less stringent.
Or, to be exact, for example, if I read my results (tubes +, +, -) acc. to Ph. Eur. than I can have less than 10 to the third, but more than 10 to the 2 second of Enterobacteria per 1 g, while reading according to USP says that the same set of tubes (+, +, -) indicates fewer than 100, but more than 10 Enterobacteria per 1 g. If my acceptance criterion is 10 to the 2 - USP says that I pass, but Ph. Eur. says that my sample does not correspond.

Why are this interpretations so different?
How do you interpret your MPNs?

Endotoxin limit calculation

I'm going to set an Endotoxin limit for a product that have two actives; lets consider that active A has an Endotoxin limit NMT 1 EU/mg and active B has Endotoxin limit NMT 2.5 EU/mg and the maximum recommended human dose for the finished product is given as 150 mg/day of active B, so can I set the same limit of active B even it is larger than Active A limit or there is any other factors should be considered?

A1: You don't have to look to the endotoxin limits of your active compounds for calculation of the endotoxin limit for your END product.

The endotoxin limit of your END product depends on 2 factors:
1: the accepted IU/kg of body mass for the route of administration.
2: the maximum recommended bolus dose in mg/kg of body mass or ml/kg of body mass

You can find the calculation in the Ph.Eur (5.1.10, supl 6.6) and USP to calculate your endotoxin limit of your end product.

My explanation is according to the Ph.Eur (sorry we only use Ph.Eur and I know that the Ph.Eur guideline is a little bit different from the USP).

First you need to find out the route of administration of your end product(intravenous or intrathecal for example). If you know that, you know the K value (IU of endotoxin per kg of body mass).
Then you have to find out what your maximum recommended bolus dose of product per kg of body mass is, the M value.

For 2 examples:
1)
A non-intrathecal drug product that has a bolus dose of 10 ml/kg
K= 5.0 IU/kg (non-intrathecal)
M= 10 ml/kg

K/M= 5.0/10 = 0.5 IU/ml

2)
K= 5.0 IU/kg (non-intrathecal)
M= 0.0143 mg/kg

K/M= 5.0/0.0143 = 350 IU/mg

Potency of the product is 1 mg/ml,
so 350 x potency in mg/ml= 350 x 1 = 350 IU/ml

You have 2 active compounds in your END product. For example: your endotoxin limit of compound A= 100 IU/ml and B= 200 IU/ml, you need to use the limit of compound A (lowest limit) to guarantee the safety of your product.

A2: If both actives are in one dose so you have to for lower

A3: You ask a very good question. It is important that you separate the endotoxin limit for the finished product (which includes the two actives - and probably some excipients) from the in-process limits for the product components, which includes the actives.

1. Calculate the finished product limit based on the dose of the whole product (ignore the doses of the two actives for now), just as you would for a product with single active. This will give you a limit per unit of dose (mg, mL etc.)

The calculated endotoxin limit applies to the whole product, actives and excipients. This is true regardless of whether there are one or more actives. The important point is that the recipient of the product must not be exposed to 5 EU/kg/hr or more. It does not matter to the recipient of the product which component endotoxin might come from, just that they are not exposed to a potentially harmful amount of endotoxin.

Note that this is not clearly specified in the Pharmacopeial Bacterial Endotoxins Test (BET) chapters or in the FDA guidance documents, but it is implicit in the endotoxin limit of 5 EU/kg.

2. Do not calculate the in-process endotoxin limits for the individual actives based on their dose as if they were used alone. To do so could allow the finished product to exceed the limit when they are combined.

The simplest way to assign an endotoxin for individual components (actives or otherwise) is to determine the masses of each component of the product in the maximum dose per kg/hr and then calculate the total mass of all components. Divide 5 EU/kg by that total mass/kg to give an endotoxin limit for each components in EU per unit weight (usually EU/mg). This will assure that the total amount of endotoxin that might reach the patient cannot exceed 5 EU/kg/hr.

You can set other limits for the various active and components is you wish, but make sure that when they are added together the total amount of endotoxin does not exceed 5 EU.

Of course, in all of the above, for intrathecally administered products, the more stringent limit of 0.2 EU/kg/hr must be used instead of 5 EU/kg/hr.

Finally, limits should be states as "less than" rather "than not more than (NMT)". The USP BET makes this clear even though USP monographs state limits both ways (but primarily as NMT). To use "less than" is safer/more conservative.

Neutralizing agents for Clindamycin and benzoyl peroxide

Does anyone have experience working with the following two materials?

1. Clindamycin
2. Benzoyl peroxide

I would appreciate if anyone has any suggestions regarding neutralizing agents that worked for neutralizing the antimicrobial properties of the above mentioned materials as per USP <61>, and <62>.

Although USP mentions that if no suitable neutralizing method can be found, it can be assumed that the failure to isolate the inoculated organism is attributable to the microbicidal activity of the product. And gives additional guidance as well. However, I wanted to get the input of PMF members who have experience with these materials.

A1: For Clindamycin Beta lactamase (Genzyme)

USP <61> Suitability of Counting Methods in Presence of Product

Hello All,
I am currently researching how companies perform this suitability method.

We are interested in using lyophilized QC strains to eliminate some of the necessary dilutions required to achieve the final inoculum concentration of less than 100 cfu in the diluted product while conforming to the inoculum volume of 1% to product volume.

We are unsure however if any company exists that make it possible so that we can inoculate 10mLs of diluted product with 100uL of a reconstituted organism (eliminating the serial dilution step when using high concentration cryopreserved organisms) and still achieving the USP standards

To help visualize:
1:10 dilution of product is used generally, (1:20 and 1:30 if acceptance isn't met initially). 10mL product in 90 mL TSB split out into 5 test tubes containing 10mLs for each suggested QC strain (*A. brasiliensis, C. albicans, P. aeruginosa, S. aureus, B. subtilis*)

We have a stock of cryocultures stored at -70C that are generally around 10^6-8 CFU /mL concentration, we then have to serially dilute these down in order to get to an acceptable level to inoculate the test tubes containing product. We then inoculate the product test tubes with 100uL of the 10^3-4 CFU / 1mL serial dilution to achieve the 10^1-2 CFU final concentration in diluted product. We then use the pour plate method with 1mL aliquots to enumerate the organisms.

Any help in making this easier by using prepared lyophilized cultures or any other suggestions you can make would be greatly appreciated.

A1: EZ-CFU by Microbiologics
Or

Quanti-Cults by Remel

Both have all organisms listed, and the end result is an inoculum or less than 100 CFU per 100uL.

A2: Yes, standardized suspensions (E-power pellets from Microbiologics) are available that allow using 100 ul of hydrated pellet of appropriate dilution into 10 ml of product.

Also, in-house suspensions can be used using optical density to estimate the concentration of your stock suspension. [name redacted] white paper can be used as guidance.

A3: We have a product at MicroBioLogics(r) that can help you. It is a quantitative, lyophilized product called Epower(tm).

For the A. brasiliensis, C.albicans, S. aureus, and B. subtilis we have an E3 Epower(tm) product available for the strains that you need for the USP <61> suitability test. An E3 product contains 1000 to 9,900 CFU per pellet. For P. aeruginosa, we have an E4 Epower(tm) product available. An E4 product contains 10,000 to 99,000 CFU per pellet.

This is how it works. 1) Dilute your product 1:10 (10 mL product plus 90 mL diluent). 2) Divide the diluted product into 5 tubes of 10 mL each. 3) For A. brasiliensis, C. albicans, S. aureus and B. subtilis, hydrate one E3 pellet in 1.0 mL phosphate buffer (There will be 1000 to 9,900 CFU per 1.0 mL) 4) Add 0.1 ml (100-990 CFU) of each microorganism strain to 10 mL diluted product. This will result in 10 to 99 CFU per ml of diluted product. 5) for the Pseudomonas aeruginosa, you will need to hydrate the E4 pellet in 10.0 mL phosphate buffer. (There will be 1000 to 9,900 CFU per 1.0 mL.) Add 0.1 ml (100-990 CFU) of the microorganism strain to 10 mL diluted product.

The microorganism suspension does not exceed 1% of the volume product, since only 0.1 mL of microorganism suspension is added to 10 mL diluted product.

A4: I throw in single shot Bioballs :)
They have a volume of about 25 µL so sample volume can be much lower
and added inoculum is very reliably 30 CFU.

Medical Device - Antibacterial barrier and Sterility test

1. What type of testing is required to claim a medical device an "antibacterial barrier"?
2. If a medical device can not be validated for sterility test because of its antibacterial properties how to establish the irradiation dose for the medical device per ANSI/ISO which needs to test the device for sterility at the end of dosing cycle? How can we claim the medical device sterile in absence of a sterility test and a dose audit?

Interpretation of USP <61> sample size

I have question regarding sample size requirements for the suitability of the counting method. Due to a limited supply of water soluble capsules for conducting the suitability test, is it OK to use less sample size such as 1 gm diluted 1:10 for membrane filtration and still use a larger sample size such as 10 g diluted 1:10 for the actual product testing?

The concentration of sample to buffered diluent is still the same however the sample size between the suitability portion and product testing is different.

FYI: This is a clinical stage product

A1: I am assuming these are unfilled gel caps. Unfilled gel caps typically are not neat validation. However the validation or suitability must be done with the same sample size as well as the same dilution used in the test procedure.

Gel caps must be tested by pour plates as they cannot be filtered.

Challenge testing of a hand sanitizer

I have been asked to investigate the need for and potential method(s) of challenge testing a hand sanitizer gel with 63% alcohol.

I am concerned that our current method of microbial testing (1:10 dilution in 3% Tween 80, saline broth) would be insufficient to inactivate the inhibitory effects of the alcohol. In addition, I have been given a very short timeline on this project, so I do not have the time or resources to experiment and determine inactivation effectiveness.

This is a new area (alcohol products) for me to deal with, and so I am hoping that someone here can share their experience and insight.

A1: We've had success validating a hand sanitizer neutralization which contained 70% (minimum) IPA using a 1:10 dilution in TAT broth with 4% Tween 20. In fact, dilution is stated in the USP as a known neutralizer for alcohols. Chapter <1227> (USP-32 NF27) is the resource to use.

A2: You have to perform neutralizer Validation before going for a challenge test. You can use following method for your intended purpose. One has to document the neutralizer efficacy validation data for challenge study reporting. You can conduct time kill study or Glove juice technic sampling study on your product (challenge study) depending up on feasibility.

I suggest you following method for your Neutralizer validation study.

ASTM E 1054-02, formulationsStandard Test Methods for Evaluation of Inactivators of Antimicrobial Agents.

I suggest you following method for your Hand sanitizer efficacy study.

ASTM E 1174 - Standard Test Methods for Evaluation of Effectiveness of Health care personnel or consumer handwash.

You can try Deyenglye neutralizing broth or try adding 0.5% soyalecithin and 4% tween 80 to your buffered broth for neutralization.

A3: use the method of European Standard EN 1500 (hygienic handrub) and for virus activity EN 14476.

sterility test method validation

Can you share with me what is the current industry practice is for validating microbiology test methods during early phase (1/2) development?

There are some discussions going back and forth whether one lot tested one time is sufficient for Phase 1 or 2 or whether the lot needs to be tested in triplicate when only one manufacturing batch is available for method validation.

Certification of Outside Testing Labs

Are there any guidance documents on certification of outside laboratories?

Suitability in Triplicate and Suitability Sample Reduction

I am trying to ascertain how necessary it is to perform USP <61> and <62> Suitability testing in triplicate, as suggested in USP <1227>. And, just to clarify, by that I mean three independent sample preparations, not the Test, Peptone and Viability Groups. I am assuming the latter is a given.

Mostly, I'd like to know what others have done and if they have received any response from FDA auditors about it, particularly if Suitability was not done in triplicate.

On a related topic, I have several questions concerning one text I've consulted that suggests to "Reduce product amount for validation work only, maintaining the ratio of product amount to volume of TSB". (For example, for E. coli, to prepare a 1:10 dilution by combining 0.1 g of sample and 10 mL of TSB)
Is this an acceptable practice and is it used extensively?
Is there a compendial reference for it?
Is there a minimum amount that can be used?

Steam quality

I've heard many conflicting opinions about whether it is necessary to test for the steam quality parameters - dryness, superheat and non-condensable gases.

Are these tests really required in a pharmaceutical facility? Why, or why not? What's the usual frequency & sampling point (i.e. most distal)?

A1: If you are dealing with Europe, EN285 requires steam quality testing for autoclaves.

Bottle effect

In the discussion of purified water sampling and testing the sample storage conditions and time are highlighted.

The assumption is that the counts will change, i.e. increase or decrease when a sample bottle is stored at ambient temperature.

Perhaps this issue needs to be re-examined.

In a recent peer-reviewed article in Appl. Environ. Microbiol Feb 2010 76 (4) 1278-1281 entitled Critical evaluation of the volumetric "bottle effect' on microbial batch growth the authors analyze the impact of surafce-to-volume ratio on final bacterial concentrations after batch growth using plate count, flow cytometry and ATP measurement and found no evidence of the much reported volumetric bottle effect.

A1: I did the test myself using transperent & brown bottles, I have found that :

1- Amber bottles gave better result than transparent ones for all microbes I used.

2- I inoculated two groups of the same bottles containing DI water with microorganisms, first group I counted the number of microorganisms per ml promptly.the other group, was kept in refrigerator for 2 hours counting No. of organisms per ml. I found that number of all organisms decreased after two hours even were kept in refrigerator.

My conclusion:

1- Amber(brown) bottles more better than colorless for sampling water.

2- Try to test water sample as soon as you can.

A2: What were the comparative numbers? Why refrigeration? What stats were performed?

A3: I have one question about this discussion. Please, could you explain to me, what is the reason for decreased number effect of all microorganisms after two hours storage in refrigerator??

A4: Besides the concentration of organisms used for your test, what was the type of microbial species that you had used to conduct the test? I have found that survivability of different types of microbial species can differ from one another when they are in just water. I find it hard to believe that the bottle composition would have a drastic effect on the number of surviving microorganisms in a water sample.. Besides, it is a common practice in most Microbiology Laboratories to analyze water samples for microbial content within 1 hour of collection to the avoid the potential of die-off of the microbial bioburden that is present in collected samples. Refrigeration of water samples (e.g. <10C) is usually only done if there is no Microbiology Laboratory on the collection site and is used during transport to a laboratory for processing [See Section 9060 B. Preservation and Storage in Standard Methods for the Examination of Water and Wastewater, 20th edition].

A5: I attended a 3 day course on maintenance of pharmaceutical water systems back in 1997-1998 and one of the presenters, I don't recall his name, said he had done studies on water samples. He found that even under refrigeration, the numbers decreased with a water sample because within 2-3 hours bacteria began attaching to the sides of the glass bottles. He said that within that time your counts could drop by as much as 1-log due to the attachment, and even with vigorous shaking, the organisms would not detach. When he went back into the bottles with a sterile cell scraper he was able to recover the organisms.

Peptone Control Group

Is it acceptable to not run a Peptone Control Group during product Suitability testing for USP <61> and <62> if the method includes the use of a neutralizing technique such as further dilution (>1:10), membrane filtration or the addition of chemical neutralizers?

USP <62> Bile Tolerant Gram Negative Bacteria

How do you interpret the USP <62> Bile Tolerant Gram Negative Bacteria Quantitative Test?

* Do you prepare a 1:10 dilution of not les than 1 gram of the product to be examined into TSB, incubate 2-5 hours, then remove 1.0 mL, 0.1 mL, and 0.01 mL aliquots to any given volume of MEEB or?

* Do you prepare a 1:10 dilution of not les than 1 gram of the product to be examined into TSB, incubate 2-5 hours, then prepare 1:10 serial dilutions into MEEB or?

* Do you prepare a 1:10 dilution of not les than 1 gram of the product to be examined into TSB, incubate 2-5 hours, then prepare serial dilutions into MEEB to obtain a final product concentration of 0.1, 0.01 and 0.001 g or mL?

A1: Could someone explain the rationale behind the quantitative test for Bile tolerant Gram negative bacteria? If the specification is the absence of the organisms, then why are we trying to quantify with dilutions? One thought I had was that you would perform the quantitative portion IF you had growth of the organisms to determine the MPN. Any input is appreciated!

A2: That's what we're doing. We perform the quantitative test only if we find the bacteria. We don't usually have to do it because Bile tolerant gram negative bacteria are seldom found in pharmaceutical products.

A3: My next question is in regards to qualifying the MLT method for a specific product. We will qualify the MLT method for a new product we have and for the first 6 lots, will test for bile-tolerant GN, E. coli, P. aeruginosa and S. aureus per our Quality Modules. I've written a qualification protocol to test for the presence of these organisms.

My question is, Do I need to run a "positive" control along side this. It seems that would be the case (perform the same procedure but in the absence of the product to see if the method is capable of detecting the presence of the organisms). This seems daunting, for the method we have to use for a lyophilized product is to mix powder in solution, sonicate, filter and then rinse with rinsing fluid. If I need to run a positive control along side, it seems I would have to perform the same method but without the product.

A possibility would be to spike the bugs to the first enrichment medias for each respective organism rather than spiking to membrane, filtering and rinsing. I will also be performing <61> to show suitability of the counting method for TAMC and TYMC and show that the material is not static.

Micro info

I have a list of organisms I am looking for more detailed information on. Can anyone suggest reliable resources that can provide more detailed information about micro organisms?

A1: American Type Culture Collection - http://www.atcc.org

A2: The best source is what is known as ... The List of Prokaryotic Names with Standing in Nomenclature at http://www.bacterio.cict.fr/

A3: Check out the Nov 2009 issue of the PMF Newsletter - Tim Sandle wrote and excellent article answering this question.

http://www.microbiologyforum.org/PMFNews/PMFNews.15.11.0911.pdf

A4: If you need information on characteristics of bacterial species, you can find very detailed information in "Bergey's Manual of Systematic Bacteriology, Second Edition".

Links to microorganism information can also be found at the Life Science Directory from the Swiss Institute of Bioinformatics. http://www.expasy.org/links.html. Scroll down to bacteria. If you need molecular information, you can find information at GIB, Genome Information Broker. http://gib.genes.nig.ac.jp/.

gamma irradiation validation

We are trying to validate an sterilization process for one of the APIs we manufacture using gamma irradiation, and we have two problems when we try to follow ISO 11137;

- first, our non sterile products usually have no detectable microbial contamination, because they are manufactured using organic solvents, so we don't meet the initial requirement (batches used in the validation must be demonstrated to have microbial contamination in al least 17 of 20 units); do you think we should contaminate the product? use bioindicators?

- second, as we pack in 1 kg units but we have to study a lot of samples in the establishment of the irradiation dose, we have to use a sample item portion (SIP) which is very different in size from the final packaging, so the correction of the irradiation dose is such that at the end we have a very high dose, that would cause significant degradation of our product.

Does anybody know how to manage radiation validation studies with APIs produced in bulk? Does anybody know a different guide on this issue?

A1: The first item you mentioned is a test used to substantiate the SIP chosen. You might work with a lab to develop a bioburden test that may detect some contamination. Bioburden for validation is performed on 10 items. Do not use BIs...try a VDmax approach that has verification dose selections at very low bioburden levels. Secondly, I suggest you irradiate in final packaging. I'd have to know more about the product to help further.

A2: Have you actually performed the validation test that you mentioned in which 20 units are sterility tested? Did you perform a bacteriostasis/ fungistasis experiment along with this test? The B/F test is very important on such a low bioburden product.

I do not believe the SIP validation test (testing 20 products) is necessary when you are using an SIP = 1.0. Can you use an SIP of 1.0?

I agree with [name redacted] (previous Form email), you should test in final package form, I would suggest worse-case scenario. As Anne mentioned earlier, bioburden testing can be performed in different ways and some ways detect bioburden better than others. These always should be validated.

Biological indicators are definitely not to be used for gamma radiation sterilization. These were used for a short time a long time ago and they were found to be ineffective.

A3: the problem with using a VDmax approach is degradation of the product and, regarding selection of SIP, our main problem is that our final package size is 1 kg so, if we want to exactly reproduce the packaging in the validation studies, we should use 1 kg samples, and that implies a lot of many and also problems for sterility analysis.

Endotoxin Limits

A1: Your finished product is adenine (API) with the additives sodium citrate and sodium acid phosphate?

Please look at the USP/BP/Ph.Eur for the endotoxin guideline.

The endotoxin limit of your finished product depends on 2 factors:
1: the accepted IU/kg of body mass for the route of administration (non-intrathecal or intrathecal for example).
2: the maximum recommended bolus dose of your product in mg/kg of body mass or ml/kg of body mass.

You can find the calculation in the Ph.Eur (5.1.10, supl 6.6) and USP/BP to calculate your endotoxin limit of your end product.

My explanation is according to the Ph.Eur (sorry we only use Ph.Eur and I know that the Ph.Eur guideline is a little bit different from the USP).

First you need to find out the route of administration of your end product(intravenous or intrathecal for example). If you know that, you know the K value (IU of endotoxin per kg of body mass).
Then you have to find out what your maximum recommended bolus dose of product per kg of body mass is, the M value.

For 2 examples:
1)
A non-intrathecal drug product that has a bolus dose of 10 ml/kg K= 5.0 IU/kg (non-intrathecal) M= 10 ml/kg

K/M= 5.0/10 = 0.5 IU/ml

2)
K= 5.0 IU/kg (non-intrathecal)
M= 0.0143 mg/kg

K/M= 5.0/0.0143 = 350 IU/mg

Potency of the product is 1 mg/ml,
so 350 x potency in mg/ml= 350 x 1 = 350 IU/ml

A2: BET limits for these shall depend on the dosage and formulation, refer to BET Guideline published by US FDA (December 1987) and shall clarify most of your queries.

A3: If no Endotoxin limit exist for your product you must calculate the value yourself using the formula:

K / M

Where K = Threshold pyrogenic dose of endotoxin per kilogram body mass in a single hour period

K is a set value of 5 EU/Kg for parenteral products and 0.2EU/Kg for intrathecal products. Other rarer values are available for other administrative routes.

and M = Maximum recommended dose of product per kilogram body mass in a single hour period

Further information can be found in the FDA Guidelines of Bacterial Endotoxin testing

http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/UCM080966.pdf . This is an old document, but much is still very relevant

and the European Pharmacopeia Chapter 2.6.14 . This is a harmonised chapter with the USP and JP

A4: for active substances with no specific endotoxin limit in the monographs, there is a formula in the pharmacopoeias to calculate the limit on the basis of dose and route of administration. Yo can find it in chapter 5.1.10. Guidelines for using the test for bacterial endotoxins (European Pharmacopoeia) and chapter <85> Bacterial endotoxins test (USP).

A5: Do you know the maximum dose? If you have the maximum dose (per kg/per hour) you can then calculate the limit. For parenteral drugs, the limit is 5 EU/kg/hr. Feel free to send me your calculations if you would like me to check them for you.

A6: With reference to your mail, herewith below mentioned endotoxin limits

Sodium citrate - 2 EU/ mg (USFDA Limit)
Sodium Phosphate Inj. - 1.10 EU/ml (USP Limit)

MLT method validation

We have done microbial Limit Test (MLT) method validation for one formulated product . Sodium Caprate (capric acid sodium salt) is one of the formulation ingredients . It is inhibiting the microbial growth and pH of this solution is 8.0.
Please suggest how to neutralize the antimicrobial activity during MLT test?

A1: Have you tried using media with Polysorbate 20 and Lecithin as per USP preparatory testing. It would help.

A2: I think dilution of the sample is the only solution to proceed for validation in this case.

Preservative efficacy test

A1: use USP General Chapter 51 Antimicrobial Effectiveness Test or BP 2010 Appendix XVI.C Efficacy of Antimicrobial Preservation. These should do it.

A2: Do folks perform preservation testing on tablets?

A3: I do not understand why someone would want to conduct routine preservative challenge testing on tablets. Would someone please explain as to why they would conduct routine challenge testing on tablets and gel capsule products? From a microbial risk assessment viewpoint, tablets do not contain a ready source of water to allow proliferation of microbial contaminants and do not contain preservatives. However, I have heard of cases in which mold growth has occurred on some types of tablets, but the cause of this mold growth was due to the tablet binders absorbing water from the environment due to an improper package. I guess that is why sometimes your see a small desiccator in some bottles of tablets. In the case of capsules, it is my understanding that it is routine practice at some manufacturers to add preservatives to the composition of the gel capsule itself to prevent the possibility of mold growth. I can understand why someone would want to conduct a challenge test on a gel capsule to verify that the inclusion of preservatives would prevent the growth of mold.

A3: My opinion is same as [name redacted]. PET is only applicable for multi dosage forms. As far I know tablet or gel capsule is not multi dosage forms. Preservative need not added to tablet or capsule. PET is mainly required for oral liquid or semi solid preparations.

A4: Analyze USP General Chapter 51 Antimicrobial Effectiveness Test, category of products (liquids for ophthalmic, nasal, oral or topic use).

Dry ovens

I would like to ask a couple of questions about dry ovens that are used in sterile production area.

1. When a cycle in a dry oven has been validated for depyrogenation / sterilisation is it required to include an endotoxin indicator or BI on every cycle for routine monitoring (confirmation of depyrogenation/ sterilization)?

2. During validation studies if depyrogenation has been demonstrated is it necessary to also demonstrate sterilization by using BI's?

A1: 1. If a validated sterilizer/depyrogenation oven is on a regular calibration and preventive maintenance program in addition to annual re-qualification, it is recommended that periodically (at least once a month) a BI is included in each of the cycles to monitor the performance of the equipment, but not on a daily basis.

2. BIs are used to validate dry ovens only if the oven is used as a sterilizer. If the oven is to be used for only depyrogenation purposes, you only need to challenge the unit with endotoxins, and to demonstrate at least a three-log reduction in endotoxin level.

A2: In general, it is not necessary to include an endotoxin indicator or BI in every cycle for routine monitoring of a validated depyrogenation cycle. Your emphasis should be on ensuring that the depyrogenation oven or tunnel continues to be in a state of control by establishing and monitoring critical parameters established during the validation. You should perform a periodic requalification of the depyrogenation cycle to ensure that it is still in a state of control, typically on an annual basis.

When performing a validation on a depyrogenation cycle in a dry heat oven, it is not necessary to include biological indicators. A 3 log reduction in endotoxin is considered to be equivalent to a much larger reduction in bacterial endospores.

A3: Many years ago I performed validation testing of dry ovens for depyrogenation. We spiked some tubes with known amounts of endotoxin, ran the cycles, and looked for destruction of the endotoxin. We never used BI's for dry ovens.

A4: During validation/ requalification, it is essential to use bacterial endotoxin to demonstrate depyrogenation.

To demonstrate dry heat sterilization, BI to be used during validation / requalification.

Endotoxin Sample holding time

I am working on sample hold time study design for endotoxin water samples. The storage time of water samples for Endotoxin determination is 24 hours and we would like to change it to a week. I saw the recent Newsletter (Vol.15) article - An Examination of the Sample Hold Times in the Microbiological Examination of Water Samples. This article discusses about bioburden samples. Are there any article's on Endotoxin sample hold time?

A1: I do not there is holding time for endotoxin samples rather than their expire date if they are kept 2 - 8 degree.

A2: Unfortunately, there is no standard hold time for samples for LAL testing. What people typically do is to validate their storage conditions. They do this by selecting the container, time (worst case, or longest) and temperature that they will store their samples. They will then add a known amount of endotoxin (in LRW or sample) to that container and mimic the storage of the sample. When the validation sample is tested, be sure to include a positive control (spike or PC) to ensure that the sample has not picked up any interfering extractables from the storage container.

A3: I agree with [name redacted]. This is basically the general rule that I have found for most things. In the absence of guidance on standard hold times, processing times, etc., a firm must draft documented evidence that any condition, procedural parameter, etc. that they are using does not adversely affect the controlled state of the process, method etc. I've seen enough arbitrary assignment of timeframes, storage conditions, etc without the documented evidence. I would venture to say if an inspector were to ask what the hold time came from, and you said we performed a study, chances are they wont even ask for it. But at least you have something to provide should they ask.

[name redacted] approach is very solid. Draft a protocol covering maximum hold times for the various sample types you would be storing (RM, WFI, In-process samples) that are representative of the various types (or choose one that you have justified as worst case scenario). I would probably spike each with a low level, and other with a high level and show there is no appreciable difference from Time Point Zero in either direction as low and high concentrations. The key is what you define as "appreciable difference" (acceptance criterion).

A4: We have done validation studies on the hold time of water samples for endotoxin testing. It is not hard to do, but good info to have on hand.

A5: Can you inform me how much endotoxin will be inoculated into sample for holding times validation ? Which method is appropriate ?

Exactly, sometimes holding time of various samples are factor. I think
that a standard guideline should establish.

Pantoea species and LEMB plates

Recently, during a test for absence of E. coli, I got the following results: pink colonies with precipitate on MacConkey Agar, purplish-black colonies that shine green under transmitted light on LEMB agar. This all pointed towards a positive E. coli result, however there was something about the colony morphology that just made me pursue it further and I identified the microbe in house via API (RapID One strip to be exact). My result was Pantoea agglomerans.

I was wondering if anyone had any information about this organism, I have read via Bergey's and a couple of other manuals that it is a non-pathogen except in the immunocompromised. Are there any concerns I should be aware of?

Additionally, are there other organisms that cause the E. coli morphology characteristics on LEMB agar? I was under the impression that very few organisms would grow on LEMB, never mind actually produce that green sheen under transmitted light.

Any information in regard to Pantoea or other microorganisms that mimic
E. coli on LEMB plates would be very much appreciated.

A1: I suggest use another identification method to be sure of the result.

A2: [name redacted], to give a medical perspective, Panoea agglomerans is a commensal and primarily a plant pathogen. However, on account of its increasing recovery from patients due to a near universal use of genomic microbial identification, it is acquiring a status of an emerging pathogen. There are three vulnerable populations. These are people with penetrating vegetative trauma like thorn pricks, immunocompromised hosts (people with cancer, Leukemias including people with indwelling catheters, elderly,) and neonates particularly pre-term babies. Septicemia is a fairly common manifestation of the infection. It can cause a marked clinical deterioration in patients but most infection respond well to antibiotics directed against gram negatives. In some cases where a sort of immunoisolation is present (synovial joints) surgery might be indicated. A couple of interesting facts about this organism is that has the ability to acquire plasmid mediated pathogenicity island that gives it tumorigenic potential in plants. Plant scientists also consider it to have a potential for use as a biocontrol agent for plant bacterial fungal infections an application hampered by its Bio safety level two designation. A genotypic deference between plant and human strains of P. agglomerans has not demonstrated. In the laboratory the main risk in a healthy individual is probably from penetrating injury with contaminated laboratory utensil.

A3: Years ago (10 or so) I ran into an environmental isolate of this vegetation organism that showed up in a product. At that time, the Biolog gave mixed results after it showed positive for the old MLT Salmonella test all the way to the TSIA butt. I do not remember if it also gave a positive green sheen EMB but I do remember the pink colonies - at the time we were more worried about the presumptive Salmonella finding. Also at that time, MIDI gave a mediocre SIM (probably would be better now) which made it hard to 100% identify but most of the data pointed to the Pantoea and that was finally written into the Investigation. It was my belief that the source was from berries on bushes around the plant and that birds were involved (some isolates came close). We trashed the product even though, as you said, it is not pathogenic.

A4: With out looking up the organism Pantoea agglomerans I can already tell you that it must be in the Enteric family. It ferments lactose+ and the reason it makes the green sheen on LEMB plates is because it also does mixed acid fermentation. The IMVic test and triple sugar iron agar slant tubes
Are very helpful tests with minimal work. Remember the definition of a coliform is that it produces acid and gas from lactose at 37C within 48 hours. So this would most likely be a coliform too. The IMViC would differentiate between environmental coliform and enteric coliforms. Any enteric coliform would be objectionable indicating fecal contamination. Enterobacter aerogenes is an environmental coliform. My guess is Pantoea agglomerans is similar to Enterobacter aerogenes. Pantoea agglomerans is not in a general microbiology book so my guess it was a bioinfomatic renaming.

When I googled the organism I got,

Pantoea agglomerans is a Gram-negative bacterium that belongs to the family Enterobacteriaceae.

Formerly called Enterobacter agglomerans, this bacterium is known to be an opportunistic pathogen in the immunocompromised causing wound, blood, and urinary tract infections. Commonly isolated from plant surfaces, seeds, fruit (ex. - mandarin oranges), and animal or human feces.

It is difficult to differentiate easily from other members of this family, such as, Enterobacter, Klebsiella, and Serratia species. The amino acid utilization pattern of lysine, arginine, and orinthine will distinguish Pantoea spp. from the others. Pantoea is negative for utilization of these three amino acids. (See Winn, et al.; "Koneman's Color Atlas and Textbook of Diagnostic Microbiology", Sixth Edition, 2006: Lippincott, Williams, and Wilkins)

This description is a mixed bag of good and bad, it is environmental and enteric. Really what is not an an opportunistic pathogen?

I guess I would have to then look at where the organism was isolated from (raw material?) and take a closer look at the manufacturing process.

Either way this organism is adding endotoxin into the product.
I would check the storage conditions of the material and see if there is moisture involved. If the material is stored where moisture can get in things will grow.

What was the endotoxin level of the material. I think most people perform Microbial lImits testing and endotoxin testing of raw materials.

A5: Pantoea agglomerans (formerly called Enterobacter agglomerans) and is a member of the coliform group as is E. coli. The LEMB plate is a selective (Eosin Y & Methylene Blue inhibit the gram positives, although some may sneak in there as small colonies) and differential media (will differentiate lactose fermenting from non-lactose fermenting gnr), so it will grow gram negatives other than E coli. Typically E coli gives that beautiful green sheen, but on occasion, have seen some goopy looking Enterobacters give a greenish glow as well.

A6: If the quality of identification was good from the API RapId strip was good (e.g. >90% or Excellent Identification), why would you repeat the identification by using a different identification strip. I can see repeating the identification if the quality of the identification was poor or to perform supplemental biuchemnical test reactions for confirmation of the identification result.

A7: Individual isolates of several gram negative species can produce the green metallic sheen and the sheen is not universally seen with E.coli. One reference with some examples is J Vet Diagn Invest 13:273?275 (2001). The metallic sheen is a function of high acid production on lactose which allows an amide bond to form between the two dyes (eosin and methylene blue). High acid production also is causes the bile salt precipitates on Maconkey plates. I high acid producing Pantoea agglomerans isolate may very well produce the result seen. Pantoea agglomerans can be pathogenic, I am not sure this is limited to immunocompromised individuals. I do agree that a second ID method (genetic if possible) would be helpful.

A8: Recently We isolated the *Pantoea* spp. from air samples. This was confirmed by API 20E strip. This is not an unusual organism. This is reported as a common microbial contaminants in Pharmaceutical environments and confirmed by using Lipid analysis and DNA based tests.
Book Reference :
*Page No.124; Microbial contamination control in the Pharmaceutical industry
by Luis Jimenez;2004*
Refer Table.14

Pantoea agglomerans is a Gram negative bacterium that belongs to the family Enterobacteriaceae.
*Formerly called as **Enterobacter agglomerans.*

In the same book also described Distribution of objectionable microorganisms in pharmaceutical raw materials and prodcuts over a 3 year period (Refer Page No.126). The isolation frequency of *E.agglomerans* is No.1 from the products and No. 2 from the raw materials.

A9: I agree with all the people, if you performed a Blast of Pantoea agglomerans in the web site of NCBI you could see that in the Phylogenetic tree the Pantoea agglomerans is very close of Enterobacter species respected of genetic code. While more closer are in the genetic evolution more characteristics and properties have in common. But I think there are more precise identification methods by which you can confirm.

Media fill

Which microorganisms are recommended for the growth promotion test in media fills?
The medium used in the media fill is soya-bean casein digest medium.

A1: You can use the following:
- Staphylococcus areus
- E. coli
- Bacillus subtilis
- Candida albicans
- Aspergillus niger
And you can use two types of bacteria (normal flora) which isolated from the sterile production facility.

A2: You could use the same organisms for media fill growth promotion as are used for sterility test positive controls per USP. (also readily available) Another option would be to replace some or all of the USP organisms with environmental isolates of same genus, species.

A3: For media fill test standard microorganisms mentioned in the USP 71 (For Sterility) or the strains isolated from your facility (Clean Rooms used for Aseptic Filling) can be used for the media fill test. And yeah growth promotion medium is Soyabean Casein Broth.

Its all mentioned in the USP <1116>. You can refer USP 31 <1116> page number 587.

A4: For growth promotion Bacillus subtilus , E.coli , Pseudomonas aeruginosa are recommended .

A5: Growth promotion testing on media used for media fill should be performed using the compendia recommended ATCC cultures for the media, as well as selected microorganisms typically found in the manufacturing environment (e.g., air, surface, personnel, water, etc.).

A6: USP 32, volume 1, 2009 <1116>, page 613, (Operation Evaluation……by the use of media fills) refers to growth promotion using indicator organisms described in <71> Sterility Tests, Table 1, page 81. It lists the strains to be used and alternatives. They are similar to those listed in <61>, but the incubation temperatures for media fill growth promotions are different for some organisms compared to those listed for Suitability of the Counting Method <61>, Table 1.

A7: Use all the organisms as defined in the USP for the medium you are using + an environmental isolate (I would recommend picking a common one, not one you have only isolated once...) .

A8: For GPT using SCDM The following microorganisms can be used.
1) Bacillus subtilis ATCC6633, NBRC3134,CIP5262,NCIMB 8054
2)Candida albicans ATCC10231,NBRC1594, IP4872,NCPF3179,
3)Aspergillus niger ATCC16404,NBRC9455,IP1431.83,IMI 149007

Not more than 100 CFU of above ref. microorganisms within 5 passages from master seedlot to be used separately for the GPT.
Incubation not more than 3 days for bacteria not more than 5 days for fungi.

A9: I would recommend growth promotion as per USP <71> Sterility Test.

A10: The organisms described in USP 32 <71> Sterility Test are S.aureus, B.subtilis, P.aeruginosa, C. sporogenes, C. albicans and A. niger (brasiliensis). The European Pharmacopeia 6.8 recommends the same organisms, but what do you think about inoculating the medium used in the media fill with an anaerobic bacteria like C. sporogenes?

A11: I agree with [name redacted]. For growth promotion test of media fill, Sterility Test chapter can be followed where specified few microorganisms. Not only that 10 to 100 cfu microorganism should be used. Few microorganisms will be tested in media fill which are isolated from environment of aseptic area.

A12: Are the filling conditions under anaerobic conditions? If that's the case, even though its not practice what I can see, maybe your media fill should challenge with FTM? Then you would growth promote that media under the guidelines for FTM in <71>. Maybe this is just "talking crazy". Never heard such a thing, but if your process has concern for strict or facultative anaerobes thriving, then you should challenge for their presence, or more appropriate, their absence.

A13: Selection of media for MFT is very critical aspect and the quality of media such as SCDM plays a very important role.
The media should be able to support the growth of varies organisms like aerobic and anaerobic as well as yeast and mold.
Also some time because of high content of sodium chloride some media are not able to support the growth of aspergillus spp and hence these organisms go undetected in test and this can be a cause of concern.
Hence please check up whether your media supports growth of following microbes:

1. E.coli 25922,8739,NCTC 90002
2. Salmonella NCTC6017,14028
3. Bacillus 6633
4. Staph 25923,6538
5. Streptococcus 6305
6. Pse 27853,27853,9027
7. Candida 10231,2091
8. Aspegillus 16404.

Question on reagent reservoirs

We use sterile individually wrapped reagent reservoirs to pool reconstituted LAL reagent for Kinetic Endotoxin test method. I observed tiny black particles in the reservoirs, which I think could lead to invalid assays. I was thinking of introducing a rinse and dry step (rinsing reservoir with LAL reagent Water) prior to use of the reservoir. Will this be a good practice. Any suggestions will be greatly appreciated.

A1: You can always just use the lid of your 96 well plate to pool the LAL reagent, avoiding the reagent reservoir altogether.

A2: We have tested a lot of reagent reservoirs and have never found one that is free of detectable endotoxin AND beta-glucan. My suggestion would be not to use reservoirs for LAL testing. Leave the LAL in the original vial and add it to the plate or tube with a repeat pipettor.

A3: I agree. That's what we do and as well as not introducing another container for your reagent I find it easier to use the repeat pipettor then a multipipettor - it's just as quick!

d values

Can anyone point me to a contract laboratory that has a Bier vessel to do D value determinations on spore strips?

A1: Nelson Labs has a BIER vessel as well as Sterigenics Lab in Shaumburg, IL., I think.

A2: Raven Labs

8607 Park Drive
P.O. Box 27261
Omaha, NE 68127

A3: we perform D-value verification of steam sterilization BIs and our BI manufacturer recommended us to work with Presque Isle Testing Laboratories//. Our contact person is:

Cindy Boris
PRESQUE ISLE TESTING
3804 Westlake Road
Erie, PA 16505
Tf. 00 1 814 836 9909

A4: NAMSA offers dvalue determination for EO, steam, dry heat and H2O2.

A5: Chris, Here at Raven Labs we have been doing ISO/AAMI/USP compliant D-Value studies for the past 10 years. We have 3 compliant steam BIER Vessels due to the large number of customer requests for resistance testing and can usually provide a reasonable turn-around time for work completion.

A6: USP describes that D-value verification by the user can be considered. In my opinion, if you safeguard the shipment conditions regarding temperature AND RELATIVE HUMIDITY, the D-value on the CofA provided by the manufacturer can be accepted. Off course these parameters have to fall within the limits as recommended by the manufacturer.

If you then choose after all to have the D-value verified by an external test lab, make sure that you record again the temperature and relative humidity during shipment of your samples.

Especially relative humidity falling out of limits can change D-values. Too much moist can trigger spores to germinate, diminishing their resistance to the sterilization (or decontamination) cycle which they are developed for.

Too dry storage can result in tougher spore with higher D-values.

I agree on the fact that verification of the D-value by the user is overdone, as long as the supply chain for your indicators is monitored.

LAL, Endotoxin and the USP BET

Most companies will not release SOPs through document control policies. You can read chapter 85 of the USP for the BET and specifics on how to do the LAL test. If you go our website, you can learn how to do a preliminary test in order to characterize your sample.

http://www.acciusa.com/pdfs/newsletter/LAL_Vol.14No.1.pdf

The relevant product insert will also give you more details.

A1: There are several consultants on this list that can provide and ensure your needs. I am quite sure that they can help provide quotes for their services. These individuals are typically the same experts that attempt to answer the questions freely.

Autoclave referral

I am in need of a *benchtop* autoclave for the microbiology lab. I am looking for one that can maybe hold ten to fifteen 500 mL or seven to eight 1000 mL media bottles, keypad input, and data recorder/printout capability. Can anyone refer me to a company that is reputable and has a location in the US?

A1: Please look at the following websites -
http://www.laboratory-autoclaves.com/
http://www.tuttnauer.com/

A2: We have an autoclave of Astell UK make, having their US division. The model of 150 litres with CFR compliant recorder and ticket printer may be useful.
www.astell.com may be tried.

A3: My company just purchased a Systec DX-100 Autoclave from Microbiology International. It will hold about 8 1000mL media bottles. Its a very nice unit and Microbiology International offers installation (water, compressed air hook up) and validation packages.

I searched a long time before deciding on this unit, large benchtop autoclaves are hard to find.

A4: Look at Sanyo. They have portable autoclaves on wheels with those capabilities. US operations are based in Chicago with sales and service everywhere.
sanyobiomedical.com

A5: Brinkman/Tauttnauer has an excellent one that will fit that need. You can easily purchase them through Fisher or VWR. They are easy to use, validate, and you can easily work it yourself should something get clogged or malfunction.

A6: Steris Corporation or Getinge come to mind. Steris is headquartered in Mentor, OH. Getinge is a Swedish company, but they have an US facility in NJ. I believe both of them have benchtop models.

A7: We have used Brinkmann/Tuttnauer for several years. It is a good autoclave that works great with liquuid cycles, however is unable to do dry cycles. Our particular model was able to accommodate about nine 2L bottles if I recall. We purchased it originally through VWR.

negative control - media fill

Can anybody tell me how to prepare negative control to observe incubated media filled vials against for any growth.

As per me incubated vials itself are negative control and no need to prepare any extra negative control.

Is this judgement ok or any specific guideline telling about preparation of negative control to observe the incubated vials.

A1: You are right. As long as the visual inspection of the vials is being done by the qualified microbiologists, I don't see any reason to think about the negative controls.

sampling tests

What protocol can be designed for a sampling test to be carried out by undergraduate students mini project work to be completed in 4-6weeks on locally sold medicaments, such as liquid syrups or tablets that can be got from a pharmacy?

A1: Try the USP Preservative Effectiveness Test.

Acceptable Humidity levels in a sterile testing suite

Does FDA dictates acceptable levels for relative humidity within a sterility testing suite and what are they if any?

A1: Relative humidity level of 30% to 50% is usually recommended for for a sterility testing suite.
The recommendation is based on personnel comfort and reduction of particulates shedding. The range ensures personnel comfort when completely gowned. The low end of the range minimizes operator shedding of dry skin, and static dispersion of particulates. The high end of the range prevents condensation and growth of mold and bacteria.

A2: Can you please send me the reference for this specification?

A3: Relative Humidity 30% to 50% is usually recommended , Temperature inside the sterility testing area also important, the temperature with in 20 to 25 °c.

A4: Please check ISPE Pharmaceutical Engineering Guides for New and Renovated Facilities - Vol3: Sterile Manufacturing Facilities. The range applies to Aseptic Processing Area as well as the Sterility Testing Suite, since the environmental controls for both areas should be equivalent.

A5: We have checked in the Guide that you mentioned but there are no specific numerical values for humidity for Sterility Testing Suite.

Fabric Sanitization

Looking for any advice on fabric sanitization methodologies. Currently looking at steam, ozone, UV....any others out there that anyone has any experience with?

prepare environmental isolates to obtain 10-100cfu/0.1ml

I am doing a bioburden validation. I was asked to challenge recovery with environmental isolates besides QC strain listed in USP.

Anyone knows how to prepare environmental isolates so that i can obtain 10-100 CFU per 0.1mL?

A1: Start with a preliminary study on your isolates: You can grow your strains overnight, or for a fixed timeline, then perform serial dilutions of the turbid culture to see which dilution will yield 10-100 cfu/mL. Alternatively, you can use a turbidimeter (or Spectrophotometer) to make a 50% turbid stock (or a certain OD600), then perform serial dilutions of your stock to see which dilution will yield 10-100 CFU/0.1 mL. Per organism, perform this a few times on separate days (at least 3 times) to get a good feel for the method that will consistently give you the CFU numbers you need (the key is to perform the test as consistently as possible in order to get reproducible data). Once you have the preparation technique figured out, you can make a large batch of your 10-100 CFU/0.1 mL isolates and freeze them in small aliquots which you can then go back to and use over a period of time for various tests.

Alternatively, you can ask companies that prepare and sell the USP QC organisms to costume make your isolates for you, but than tends to get pretty pricey!

A2: This will take some attempts at serial dilutions to target the correct inoculum. I used to prepare growth promotion cultures of USP organisms before the pre-calibrated products were available. What you will need to do is get a loopful the organism from a plate and transfer to the appropriate media (probably TSB). Let it grow for 24-48 hours (30-35C). Take a loopful of this and transfer to a fresh TSB tube. Incubate 24 hours (don't vary too much from 24 hours exactly, as the growth rates of some organisms are very fast). Most organisms after 24 hours this way will be between 10^6 and 10^8 cfu/ml (I would keep this 24 hour TSB culture refrigerated and subculture from there next time you need to prepare the inoculum if you plan to use the organism regularly). Perform serial 1:10 dilutions using sterile buffer. Since you don't know which dilution will yield the correct concentration, you will need to plate several dilutions the first time. I would plate 0.1 ml in duplicate of probably the 10^{-3}, 10^{-4} and 10^{-5} dilutions. Incubate the plates if you are lucky, one set will be between 10 and 100 cfu. If not, you will probably need to adjust the first dilution to get it right - for example 3 ml culture to 7 ml buffer or similar adjustment. Once you have the correct scheme, it will work pretty much every time as long as you subculture one loop to fresh TSB 24 hours prior to preparing the dilutions. Hope that helps.

A3: Hi, Here is a one way to do this:
1.You would grow up the environmental organisms per appropriate procedures (optimum growth temp and time differs for each organism).
2. Then determine if that culture was pure, if yes then you would identify it for confirmation.Identification that yields at least a 99% confidence should be used for preparation of master stocks and EM isolates etc.
3. Perform a turbidimetric reading (take OD of the culture), compare it with a McFarland Std 1 and 2 such that the OD of your culture lies between OD of Mcfarland Std 1 and 2 or is equivalent to the mean of the two ODs (McFarland Stds 1 and 2). For ex: McFarland std OD = 0.2 and that of McFarland Std 2 = 0.4, your culture should yield an OD value between 0.2 and 0.4 .An OD value closer to Mcfarland Std 2 is much better, that way you start off with a higher cell count which you can always dilute down. Mcfarland stds 1 and 2 correspond to an approx. cell density of 3 x 10 8 and 6 x 10 8 cells/ml respectively.Say your OD value is 0.213, your cell density would approx. be equal to 6 x10 8 cells/ml, with processing errors and cell death accounted for , you would end up with an approx. cell count of 6x 106 cells/ml.
4. Assuming your starting conc. of this culture is 6x 10 6 cells/ml, perform a 3 fold dilution using TSB with glycerol (cryoprotectant) ..This should bring the cell count approx. equal to 6000 cells (if your starting conc was 6 x 10 6 cells/ml).You can dilute this count further to yield an approx. cell count of 100 cfu.
5 Perform serial plating in duplicate or triplicate to confirm and to arrive at the 100 cfu count.
Hope this makes sense...
I did this for all my EM isolates in my organization. Used this method for fungals and bacteria as well. Remember if you plan on making 100 vials of 0.5 each, remember to perform the dilutions and adjust the volume accordingly.

A4: Quantify the isolate to about 100 CFU/ml. A little more than 100 CFU wouldn't be a problem. Take 0.1 ml of the suspension which will contain about 10-100 CFU.

A5: If your QC micro Lab uses the bioMerieux VITEK System for microbial IDs, you can use the colorimeter from this system. You can also use a standard spectrophotometer in the chemistry lab.

First, grow up your environmental isolates on an agar plate for 18 - 24 hours or as appropriate. Suspend enough of the microbe to make a 0.5 or 1.0 McFarland standard suspension in the appropriate diluent for your test. You use the colorimeter to make the McFarland standard equivalent.

Prepare 10 fold serial dilutions (usually 10 -1 to 10 -8 or -9) and determine the titer (CFU) of your suspension. You probably want to do the titration in at least duplicate, triplicate is better. Average your counts.

Use this average titer to determine the dilution you will need to make to yield 10 - 100 CFU per 0.1 mL for your tests.

This method gives you a robust, non-stressed, well growing microbe for your tests.

Grow up your bug, make up the McFarland equivalent suspension and make the appropriate dilution before each assay.

A5: You can culture your environmental isolates in a broth (TSB for instance).
After overnight of (rocking) incubation, you aliquot the TSB in freeze vials. These vials are then frozen at -70°C. There you have a cell bank.

This cell bank can be qualified: for instance, you take out 3 vials and thaw them at room temperature.
From these vials, you prepare dilution series in sterile saline 0.9% solution.
From every dilution, you spread 100 µL on agar plated media and incubate.
After counting the colonies (30-300?), you determine the amount of CFUs per mL in your original aliquot (CFUs x dilution x 10).

Describe your colonies, check for purity and microbial identify your organisms. Then you are USP compliant.

When preparing a challenge for suitability testing of your microbial limit test, you calculate from the determined titer a dilution yielding 500 CFU/mL. If you take 100 µL of this dilution, theoretically it should contain 50 CFU/100 µL. Right in the middle of <100.
This spike can be used in your suitability test. For control, you spread 100 µL of the dilution on applicable agar plates and incubate to prove the spike being <100.

You can expect variability and therefore it is wise to test with also other dilution yielding some more or some less CFU.

growth of organism for media hold

Does anyone know of a sound reason why I would get growth for a media (NMB2) inoculated with S. paucilimobilis when it is prepared on a small scale but then not get growth when it was made on a larger scale in a vessel?

I inoculated both solutions with <100 CFU.

A1: In a previous life, I used to work for an old media maker - Scott Labs in RI.

You need to remember, that even though you may not have changed your actual sterilization cycle time, the larger vessel may take a lot longer to heat up and cool down the media.

This extra time at higher temperatures (especially on the cool down side) can destroy essential, temperature sensitive nutrients, importantly those present in low quantities. Therefore, the formulation of your media may need to be adjusted.

A2: I'm not familiar with the phenomenon in this context but saw it in a fungal context. Rationale offered was that siderophore production by the strain was limited so the exponentially greater dilution in large batch culture left the inoculum effectively starved for iron.

A3: Are you using a validated cycle for the vessel or you are using an overkill cycle? An overkill cycle may be overcooking your media in the vessel and destroying essential nutrients required for growth of S. paucilimobilis. Secondly, do you have a validated cleaning and sanitizing procedure that assures a complete removal of traces of detergents and/or sanitizing agents from the vessel before vessel is used for media preparation? Detergent and/or sanitant residues from the vessel may also affect the growth of the organism in medium prepared using the vessel.

A4: Do you have the same inoculum concentration in the larger vessel than in the smaller one? If yes, it may be a lower dissolved oxygen supply or less efficient agitation (mass transfer) in the larger vessel. What are the vessel volumes?

A5: I have a lot of experience with sterilizing bulk media from a previous job in a biologics facility that made most of their own media in 20-liter or 50-liter carboys. In that facility, we found that using an F0 controlled cycle worked best because of the long heat-up and cool-down times for the media. Using a traditional time at temperature cycle for the media caused significant problems with over-heating of the media and failure of growth promotion.

What is the safest method for disposal or decontamination of used media

What is the *safest* method for disposal or decontamination of used media.

After Decontamination cycle (autoclave).What disinfectant may be used, which is less harmful to the Microbiologist.Presently i am using *Phenol*.

A1: If you have sterilized the used media with a validated autoclave cycle then there is nothing special about the trash. After all, media is food. If you have used some particular chemicals such as cyanide then chemical   precautions are necessary.

USP<61> test

I have very simple question. To perform the total yeast and mold count which agar you are using plain SAb or SAB with chloramphenicol. Since as per USP any growth on SAB plate is considered as a part of Total yeast and mold count. Which agar is more desirable?

A1: It would depend on the application. If your sample normally contains bacteria and you want to inhibit bacteria growth use Sab Dex w/Chloramphenicol. On the other hand if bacteria are typically not present (pure culture) or in very low levels, Sab Dex will suffice and generally be less expensive and more readily available.

A2: If you are encountering more bacteria in your sample then you can use SAB with chloromaphenicol or else SAB is ok.

A3: If you are finding that you have a large number of bacteria growing on your SDA, which would (somewhat artificially) increase your fungal count, then using SDA with chloramphenicol would seem to be a good idea. Whatever you choose, validate the method. And don't look for too much logic in the pharmocopoeial requirements…

A4: As many dosage forms have TCYMC limits of 10 cfu/g or mL, my lab uses SAB with chloramphenicol to eliminate bacterial colonies from the plates

A5: I can tell you one thing. Usually, You can use just SAB, without chloramphenicol additive, but if you have sample with potentially growth bacteria (like when your sample is syrup, or some other herbal sample) you need SAB with chloramphenicol, or some other antibiotics additive. On this way you remove microbial effect. Because chloramphenicol is inhibitor for wide spectrum + and gram - bacteria.

A6: I echo [[name redacted] comment and add that chloramphenicol is not without effect on fungi - mitochondrial function (aerobic respiration) will be inhibited - but it requires a greater concentration of the antibiotic for complete inhibition. Some fungi are more sensitive than others. Tho' it's unlikely you'll miss any with the antibiotic-supplemented medium, suggest you use it only if you need it.

A7:   I have found that it is common practice for many laboratories to use Sabouraud Dextrose Agar (SDA) with chloramphenicol to prevent bacteria from growing. In general. SDA has a pH of 5.6 +/-0.2. To determine a bacterial count, most laboratories are using Soybean-Casein Digest Agar Medium (Tryptic Soy Agar) that has a pH of 7.3+/- 0.2.   How do you verify or have assurance that the bacteria that can be recovered on SDA without chloramphenicol will be recovered on TSA plates?   The reason I'm asking this

question is that in the past I came across a bacterial contamination issue in which contaminating bacteria had only been detected to be present in the finished product sample by using Petri dishes containing SDA without Chloramphenicol. It was not detected on TSA Petri dishes. Upon further investigation, it was determined that these contaminating bacteria had an optimum pH for growth at 5.7. At pH's greater than 6.0, they would not grow on TSA unless the pH of this agar was lowered to be below a pH of 6.0 or on SDA Petri dishes that had a pH adjusted to be above 6.0. In the end, the contaminating bacteria for the finished product was traced back to a USP material that has an aerobic microbial limit specification of 100 CFU/gram. The counts were in the thousands on SDA without chloramphenicol, but none were found on the TSA Petri dishes. Because it has been several years, I do not remember the exact id. of the organism, but it was not a lactic acid bacterial species because it was a Gram-negative species that adapted to grow at a low pH.

A8: I agree with [name redacted], I have seen in the past Acetobacter liquifacien contamination which grew (brown colonies) acidophile on SDA without Cloramphenicol but not on TSA. The organism was found in millions in Aloe Vera drink, gave an off taste to the product. The supplier lab had ignored it because there were no yeast or molds on SDA and there was no growth on TSA.

SAS vs. SMA comparison

Has there been any studies published that compare the SMA to the SAS viable air samplers? Specifically, moving from the SMA platform to the SAS platform. Thank you in advance.

A1: Yes there is a publication and I am having it sent to you. There are good studies which show that the SMA collects 20% of the microorganisms collected by other direct impaction samplers like SAS.
The principal problem is simple physics - the large holes in its head and the suction from the air do not create enough velocity for the particles to impact on the agar, leading to false negatives for the smaller microorganisms 1-5 microns in size.

A2: There are many published studies that evaluate many of the samplers
in the marketplace. Some evaluate the samplers independently against a known control (such as the ISO 14698). Some utilize a side by side by side evaluation. And some that utilize a D50 theoretical analysis for determination of capture. I have been involved in many of these for over 25 years. The older study mentioned by Christine below had major setup problems for the SMA that definitely compromised capture in those units. When this was published we acquired as to the setup conditions and were shocked that such setup was done as it was done. The following were some of the major set up problems with the SMA.

1. An improper flow rates below 28.3 LPM was used. (14 LPM was used). The SMA requires 28.3 LPM or 1 CFM to operate appropriately in the Atrium Model and 5.0 or 1.0 CFM in the MicroPortable model.

2. An improper level of media was used. An SMA 32 mL fill unit was used with 18 mL of media. That provided too much distance between the SMA top and the agar causing loss. An SMA 18 mL would have been required to be used to see appropriate results.

3. The SMA 1 CFM (28.3 LPM) unit was compared to units running at flow rates from 90 LPM to 322 LPM. But sampling time was not adjusted appropriately to accommodate total volume sampled.

4. The SMA equipment in the study was not attained from VAI and was a partial non-workable system.

5. As the SMA is available in many styles based on what the end user desires to attain, the SMA-D50, SMA-25 and SMA-18 and SMA MicroPortable were not evaluated.

All the above skewed the results improperly. The authors were apologetic but the study was already published.

The SMA has been used for over 30 years in the pharma and biotech industry. If tested appropriately it's capture compares equally to most samplers. To accurately do this study, the above needed to be accordingly.

In review of many of the sampler comparisons many factors besides capture all add into which sampler is the best unit for operations. Many of the evaluations in the world are done by academia and not personnel working in GMP operations. And thus, critically important industry criteria are either unknown from lack of experience or overlooked. As an alternative, a few years ago I wrote a book chapter that complete addresses the requirements of microbial air sampling and compares the models for sale.

"Sampling Equipment", Art Vellutato, Jr. Environmental Monitoring, Edited by Jeanne Moldenhauer, Chapter 18, Davis-Horwitz Publishing, P219-268,2007

I can send you a copy of this or you can search for the reference below. This book chapter also discuss critical choice selection details such as:

1. The critical nature of assuring a sterilized sampler in Grade A/B areas
2. Adverse effect of some samplers on laminar flow and the environment
3. Exhaust contamination from samplers
4. How capture is derived
5. Regulatory Expectations
6. System Design

.....and much more

Steam sterilizer temperature data printout requirement

What are the requirements for the format of steam sterilization temperature printout and graph?

1)Only temperature graph is required for the cycle?
2)Temperature Graph with temperature printout with actual value(...which is showing only drain sensor temperature and pressure with FO vale ...) is required ?
3)OR Temperature graph with steam sterilizer sterilization cycle, along with temperature data printout { showing all the sensors(Temp. and pressure) ?

And what should be the ideal time interval between two readings ????

A1: Condition 3 is Ok.

A2: ph alone. But this should be addressed in your SOP and reading of thermograph shall be described appropriately. Equivalency of results between thermograph and data print, shall be demonstrated before utilizing thermograph independently in routine.
In most of sterilizer drain probe is the controlling probe, as it is found to be coldest, so data from this probe can be acceptable. If your sterilizer has PLC recording data from all probes, then nothing like it.
1 min is sufficient interval for recording cycle data.

A3: Do you need temperature graph with temp readings and F0 Value during validation or is it for daily bases.

If it is for validation then you need 12 Probes per cubic meter, temperature readings at 15 seconds interval & you will get temperature graph, & F0 values automatically.

If it is on daily basis you should have 6 probes in built in your sterilizer and the reading of 1 minute.

A4: You can go for the second option in addition to few more sensors showing the temperature in center and on top based on the design of your autoclave.

A5: I would be very interested to know the source of the stated requirements of 12 probes for cubic meter. At this rate I would need 300+ probes to validate our chambers. This can not be correct.

A6: EN 554, A.3.3 is reference for 12 Loggers per cubic meter. AAMI TIR #13, 4.2.3 and 4.3.3, minimum of five loggers per 100 cubic feet of empty chamber volume and 10 loggers per 100 cubic feet of chamber volume for loaded chamber.

Ampoule filler validation

I am finishing a project that entails the validation of an aseptic ampoule filler that comprises of an ampoule filler contained within a contained isolator environment meeting grade A limits.

I am looking at non-viable particulate counts, with fantastic results meeting grade A limits when monitoring the filling point with the machine running but no gas burners running or ampoules being opened, filled and recapped. Obviously, when the gas is burning and the amps are being opened, filled and recapped the particulate counts are going up and up - which I anticipated, but I was wondering if anyone had any experience of what sort of particulate levels to put on such a process, or even if there is any point in monitoring for particulates since these will always be present in high numbers in such a process.

Any ideas or references I could use would be appreciated.

A1: Your reference is Annex I EU CGMPs and the foot note where it says that there should be a recovery period for returning to at rest levels,

"14. The particle limits given in the table for the “at rest” state should be achieved after a short “clean up” period of 15-20 minutes (guidance value) in an unmanned state after completion of operations.”

neutralizer

I want to test the antimicrobial efficacy of a product with Chlorhexidine Digluconate. What will be the appropriate neutralizer for this chemical?

A1: Try testing by membrane filtration with multiple rinses.

A2: Polysorbate 80 and Soya lecithin in combination, are good neutralizer for Chlorhexidine. Try with different concentration of above considering the concentration of Chlorhexidine.

A3: You can use SCDA with Tween 80 and soya lecithin.

A4: To neutralize the antimicrobial activity of Chlorhexidine digluconate, anionic surfactants or a combination of a nonionic surfactant (e.g. Polysorbate) and Lecithin can be used.

Statistical significant sampling procedure for BET release testing of injectables

Can anyone advise or comment on Statistical significant sampling procedure for BET testing of injectables,

Current practice of testing one vial/syringe from the beginning of a batch, one from the middle and one from the end of a batch for LAL test does not satisfy some regulatory authorities

A1: I agree with you that three vials is not going to be enough. You must use a statistically valid sampling protocol based on batch size. The FDA Guideline does outline a sampling procedure for DEVICES which is based on lot size.

BET 3 log reduction validation from machine parts

Does anyone have any suggestions or methods on how to prove Bacterial Endotoxin removal (3 log reduction) from machine parts ,CIP systems or coupons.

Testing the final rinse for BET during cleaning validation is not sufficient for some regulatory agencies.

A1: I beg to differ. Rinse samples and swabs are recognized as equivalent.

A2: If you absolutely want to be sure that no endotoxin survived on your coupon, you can immerse the coupon in chromogenic lysate. After a suitable incubation period, stop the reaction with acetic acid, and measure the color. This will cost you a fortune in lysate, and you will need to set up an endpoint chromogenic assay to perform quantitation. That, in itself, is not going to be a fun project.

I suggest that you verify that regulatory agencies won't accept this method. It is a well established method and described in quite a few articles. It has been used by industry for years to validate depyrogenation ovens. Make sure that you are getting proper information and not someone's interpretation.

A3: The methods to use are the swab and the rinse method. . In your case try both. Validate the method on coupons first using flocked swabs. Coupons are to be spiked with an endotoxin standard. I would also validate the rinse method.

When performing the in-situ testing on equipment such as a tank, take the swab sample from the most vulnerable sample point (i.e. exit or drain valve). This would present the worse case scenario. The validation on coupons demonstrates that the validity of the method. The in-situ testing demonstrates the cleaning procedure is effective for the targeted object.
As for as using the final rinse, if there is a method validation in place that is satisfactory, there should not be any problems with any agency.

A4: What people typically do is to validate their extraction, much like one would during the validation of a depyrogenation oven. This is done by challenging the article with a high amount of endotoxin (e.g., 1,000 EU recoverable) and then putting the item through an extraction procedure. You would then show that your extraction has removed at least three logs.

Bacillus cereus

Can someone comment about the significance of isolating this organisms in drug products such as tablets or in an environmental monitoring result. Since this organisms is on the FDA bad bug list, is this now consider an objectionable microorganisms and all gram positive rods need to be identified down to the species level to eliminate the presence of this organisms? The information on this organism mostly pertain to food and the toxins it produce.

How is everyone interpreting the significance of this organisms if found in oral dosage forms or EM and is it your company's practice now to identify all gram positive bacteria to the species level?

A1: This microorganism can be a real problem depending on the targeted type of patients. Most microbes will not survive compression, but one exception is spores. The B. cereus spore coat is very thick and can germinate at 4 deg. C. From my experience with this microorganism, it is very hard to kill. When doing disinfectant efficacy, a longer kill time may be necessary.

A2: B cereus is in the FDA Bad Bug Book as it produces an enterotoxin in unrefrigerated food such as brown rice that causes a mild self-limiting intestinal disturbance. However, to produce sufficient toxin to cause vomiting there needs to be around 10^8 cfu/g.

Low numbers of B cereus in a tablet or isolations of B. cereus spores during environmental monitoring are not a major concern.

identifying all gram-positive spore formers to a species level is extreme. I would certainly identify if alert levels are exceeded to aid corrective actions.

A3: It's good practice to identify Gram positives to the species level since there are other gram positives that are objectionable as well, such as Listeria, Streptococcus, Staph, Coryneforms, etc.

The more you know, the safer your products. And the more definitively you can defend yourself and your processes.

A4: I will second what [name redacted] has said, but add the following: If isolated in EM, this particular "Bad Bug" can be a ring-tailed SOB to get rid of in an aseptic facility. Bacilli are tough and durable and it frequently takes multiple sporicidal baths before the critters can be eradicated- they are very persistent.

A5: This organism is a very potent toxin producer in some foods and is a problem organism in immunocompromised patients, sometimes causing sepsis and meningitis. There are two culture media available for isolating and identifying isolates. They are Bacillus cereus agar (Acumedia) and MYP Agar (BD).

A6: My response was to the question of why this is an FDA BAD bug, not that it is comparable to Clostridium botulinum. It is also a potent producer of food-poisoning (personal experience).

A7: The FDA Bad Bug Book addresses food-borne pathogens. B cereus is a food borne pathogen so it is in the listing that is readily accessed via the internet.

Because I disagreed with your description that it is a very potent toxin producer i compared it to C. botulinum.

Plate pouring equipment

Can anyone recommend a low budget efficient system for plate pouring using a peristaltic pump? We can buy an off-the-shelf system if the price is right but can also cobble together the components for a homegrown system. We need to pour several hundred petri plates in a run.

A1: Check out New Brunswick Scientific. The had a device called a "Platomatic" that was quite efficient.

A2: You can try Mediajet / Mediaclave - google to get more details

A3: At Probiosys center for excellence for training in pharma microbiology, at Bangalore, India we have an Integra Doseit pump which can be used aseptically for the application you have mentioned. But maintenance of temperature, addition of additives, validation of the integrated systems like tubings, vessels for stocking, autoclave, etc can be challenging. This can be used for reusable and disposable plates.
Alternatively automatic pourer and stacker from AES France, APS-one which can dispense to 90/55 mm disposable plates at 750 plates/hrat 15ml per plate can be used.
For more information you can refer www.ibsintegra.com and www.aes.com.

A4: Could someone comment on the current documentation practices for <61> & <62> growth promotion? I have noticed a wide range of documentation practices but I believe the correct way of documenting the GP test results include to document details such as inoculums used, incubation temp & duration of incubation and results (counts or other depending of media being tested). When put together, the test matrix could be complex and difficult to document if it is not well organized.

Also, I would like to see comments on the application of the growth promotion guidelines found in <61> & <62> to media used in other micro assays (i.e. air sampling media, surface sampling media, purified water media, etc)?

I would assume that for practical and convenience purposes you may want to maintain a similar approach to the guidelines found in <61> & <61> but the fact is that there are no specific guidelines requiring to do this with the media used for other microbial test (which will specify the level of detail found in <61> & <62> i.e., org to use, incubation time, incubation temp, etc).

Also, is there any value in keeping a file with the information provided by the manufacturer for each lot of purchased prepared media provided that you will have to do your own testing when received or is the general practice to do limited GP testing and maintain COA in file?

Finally,

* What is your experience in terms of man hours needed to keep up with the <61> & <62> growth promotion requirements?
* Are the requirements adding a significant amount of time prior to be able to use the media and consequently you need to keep an adequate inventory to ensure no test scheduling disruption due to media not being ready for use (qualified).
* Is some using a risk based model and using the media concurrently while executing growth promotion?

A5: [OP] Could someone comment on the current documentation practices for <61> & <62> growth promotion? I have noticed a wide range of documentation practices but I believe the correct way of documenting the GP test results include to document details such as inoculums used, incubation temp & duration of incubation and results (counts or other depending of media being tested). When put together, the test matrix could be complex and difficult to document if it is not well organized.

-Much of that information (inoculums to use, incubation temperatures/durations) can be contained in your applicable SOP. Our actual documentation allows the technician to record organism (genus/species), then passage/lot/ATCC#, incubation temperature/duration, and then the counts for population verification and test plates (followed by a % recovery).

[OP] Also, is there any value in keeping a file with the information provided by the manufacturer for each lot of purchased prepared media provided that you will have to do your own testing when received or is the general practice to do limited GP testing and maintain COA in file?

-I'm not sure how much media you go through, but a lot history packet would not take up much space. We have both the manufacturer's CoA and our testing worksheet within our Lot History packets.

[OP] What is your experience in terms of man hours needed to keep up with the <61> & <62> growth promotion requirements?

-That depends on what organisms you use. For example, environmental isolates that are cryo-frozen will require at least a few days to grow out, serial dilute, read to confirm your inoculums dilution, etc. You can buy many ATCC organisms lyophilized so that a simple 15 minute reconstitution in a buffer will have them ready to be plated in known inoculum sizes. This also depends on how quickly you go through media...you can always buy larger sizes of the same lot to not have to test as often.

[OP] Are the requirements adding a significant amount of time prior to be able to use the media and consequently you need to keep an adequate inventory to ensure no test scheduling disruption due to media not being ready for use (qualified).

-Once a steady-state exists, standing orders on media help this process immensely. I would advise always having two lots in-house…one "in-use" and the other "on-deck". The backup lot needs to be tested

as soon as possible, as if it fails growth promotion testing in the week before it is needed, you will be in some big trouble!

[OP] Is some using a risk based model and using the media concurrently while executing growth promotion?

-I have worked where this is permitted. This did bite us on the butt at least once. It should not be necessary if the department has adequate time management. I would not recommend taking on that risk.

A6: Simply put, you want to document anything that would be needed to reconstruct the test. Thus, you need to document things like the organism used, the inoculum level, the incubators that the plates went into, as well as the time they went in and the time they came out, esp if there is a time requirement, the lots of the media, etc. Only what is documented is what can be considered to have been done.

In regard to the C of A's I would keep copies of all C of A's, both for media and for the cultures that you are using.

In regard to growth promoting media please refer to the current USP, chapter <1117> Microbiological Best Laboratory Practices, which states:

"Quality control tests should be performed on all prepared media. Tests routinely performed on in-house prepared media are pH, growth promotion, and periodic stability checks to confirm the expiry dating." (Bolding is mine).

Using media "At Risk" while you are waiting for growth promotion results can be a dangerous practice. Since microbes are not necessarily homogenous in a sample or in an environment, if you lose data because the growth promotion failed for any reason, you may not be able to reconstruct it. I would suspect that there may even be some 483 observations about this practice if you looked for them. Maybe someone else in this list will have information about this.

Gown room design

Can someone direct me to a formal document that describes gown rooms being broken up into black/gray/white areas.
Does anyone have experience with FDA audits and having a gown room that is unclassified?
Grade D or ISO Class 8 are what most gown rooms are classified?

A1: normally the gowning room is classified depending of the following cleanroom. The gowning area to Grade D/ISO 8 is ISO 8 too and the gowning room to Grade B will be classified Class B too.
I scheduled several gowning areas but I know no formal document that give really detailed regulations.

If you have a look in the EU GMP Guideline you will find more general advice like the rooms should have a conceptual design to minimize risk of failures and contamination and avoid entrance of trespassers.
In Annex 1 of the EU GMP Guideline it's a little bit more defined:
...the last area of a gowning zone should have the same grade as the following area....;
...the gowning procedure should be done in divided steps to minimize contamination risk...;
...avoid that both doors can will be opened at the same time...;
... pressure difference 10 - 15 Pascal:

But there are no definitions for the furniture etc. This is a classical "state of the art" issue. To minimize risk of contamination it will be necessary to storage unclean and clean garment in different wardrobes, to use a sit over bench, and so on.

Incubator validation

I would like to receive comments regarding incubator validation. Are there any standards for number of measuring time (continuous operation) and number of opening and closing door etc.

Any comments as always will be appreciated.

A1: It is easy nowadays to monitor them all the time with computerized monitoring. But manually we were recording it 3 times a day and it was accepted from all auditors including FDA inspectors.

About no. of opening and closing them, it should not cause out of the validated range of the incubators ie. for fungi 22.5 + or - 2.5 and for bacteria 32.5 + or - 2.5 not out of these range or other validated ranges as for faecalis E. coli or biological indicators and so on.

A2: Guidelines for incubators and stability cabinets validation can be seen in 21CFR 211.22(c)
Initial PQ- temperature mapping validation studies include empty and full chamber content studies.
Routine frequency and duration of door opening studies for 24hrs or 7 days to cover full routine usage or automatic defrost/chilling if relevant.
At least 8 sensors or thermo couples required for < 5 cubic meter chamber space.
In cold rooms 1 probe for every cubic meter is required.
Continuous monitoring preferably automatic with ticket required.
Temp. deviation limits between probes, cold spot and probes, and variation between set point and actual temp achieved to be documented for PQ.

A3: you want to do it enough times to resemble the daily use of the unit. That is the safest bet. And I would measure every minute in that case so you see the activity as the door opens and closes.

A4: I do not find these prescriptive validation requirements in my copy of the 21CFR211.22(c)

Neutralizing Ibuprofen antimicrobial activity

I am working with a client to develop a microbial limits test for bulk Ibuprofen. Do any of you have insight on how to neutralize the antimicrobial activity? I have found many articles on-line that discuss the antimicrobial properties, but none that talk about neutralizing it.
At this point they only use pour plates and are not set up to test by filtration.

A1: try with tween 80 5%

A2: For recover microorganism in pour plate, you need to performed a dilution 1:50 for Salmonella (Objectionable) and 1:250 for (E.coli and S.aureus). For pour plate method use a dilution 1:200 and plating 4 mL. You could plate 1 mL in 8 plates, sum 4 plates, average and multiply for the dilution factor.

A3: This is a reference:

Lett Appl Microbiol. 1995 Feb;20(2):82-4.
Antibacterial activity of the anti-inflammatory compound ibuprofen.
Elvers KT, Wright SJ.
School of Biology and Biochemistry, University of Bath, Avon, UK.

The effect of ibuprofen on growth in vitro of six bacterial species was tested. Ibuprofen inhibited growth of the Gram-positive species, but the two Gram-negative species were unaffected. Growth of Staphylococcus

aureus was suppressed by ibuprofen concentrations greater than 150 micrograms ml-1 at initial pH 7. At pH 6, such concentrations prevented growth. The antibacterial activity of ibuprofen was affected by pH, being more effective at values below pH 7. Ibuprofen may have an ancillary benefit in topical application, in controlling bacteria.

PMID: 7765904 [PubMed - indexed for MEDLINE]

The concentration and pH will be critical.

A4: Try to increase ph to about (7.2 - 8.0)

Some friend tried this before and gave him good result as he mentioned.

A5: we have a lot of products that contain ibuprofen, and all of them demonstrated an antimicrobial activity to some extent, in some cases with all test microorganisms proposed by the pharmacopoeias. We found out that general neutralisers, like polysorbate 80 and lecithin (for example 15 g/l lecithin and 50 ml/l polysorbate in buffered sodium chloride peptone solution) did the trick, but not at the first dilution 1:10, but on following ones, 1:100, or even 1:1000 depending on the ibuprofen content. Sometimes greater volumes of agar media help - one time we used plates 150 mm in diameter, where we plated 10 ml of the 1:1000 dilution (0.01 g of the sample), and it worked well.

A6: If you use a 1:250 dilution for E.coli.
How many mg's of product do you test for the absence of E. coli (2.6.13)?

A7: Try these dilutions with TSB Lec/T80 and follow the chapter 61 and 62 of USP.

A8: There are manufacturers who offer Soyabean Casein Digest agar with neutralizers like tween 80 and soya lecithin. You can try this one.

A9: The dilution is with buffered peptone water 0, 1%?? Use you triptone soya agar and sabouraud dextrose agar??

Cell densitometer

I am looking for a small and portable cell densitometer to estimate the concentration of cell suspension.

A1: Cell densitometer DEN-1 of Grant, UK may be useful. Ref www.grant.com

A2: try McFarland standards. Very portable, does not take any power, etc.

Help needed regarding antimicrobial level in product

I have a research question that I'm hoping some of you might be willing to assist with. I have a snow globe sitting on my desk that includes the following liquid:

84.8%: Water (H2O) - CAS#7732-18-5
15% : Propylene Glycol (C3H802) - CAS#57-55-6
0.2% : Antiseptic(C4H4CLNOS)-5-Chloro-2-methyl-4-isothiazolin-3-one - CAS# 26172-55-4

My question is regarding the 0.2% Antiseptic portion...is 0.2% a high enough concentration for this type of application??? I'm not familiar with the use of this chemical as a stand-alone agent, but know it is a component of Kathon CG and other similar products.

Now, the issue is that there are floaters/fibers/debris in the water that to me look like fungal growth or dissolved paper filaments...my testing lab results are showing Enterobacter aerogenes...so I have a bit of a question with their analysis, but I wanted to reach out to you with the question as well.

Capsule dissolution

Does anybody know a way to dissolve Gelatin Capsules such that they can be turned into a solution/ suspension that can be filtered through an 0.45 cellulose filter.

Microbiological Testing of API and Raw Materials

I will like to know the practices followed for testing of API and Raw materials. Is it done container / Pack wise or It is done on composite sample?

Bubbles in RCA plates

We're having lots of issues with small bubbles in our RCA plates which get counted on the Q-count, and cause us lots of rework. We're following standard pouring practices, but this media is giving us fits. We're looking for a solution that will prevent bubbles, versus dealing with bubbles after they form (like flaming plates). Any suggestions?

Consistency of microbial count result

Sometimes the result of microbial count is inconsistent. In first test observed 650 cfu/ml but second time in repeat test found 1100 cfu/ml for same bottle or same sample. In that case, we have to face in critical situation. Decision making is difficult that how many cfu content actually in that sample.

A1: In Kinetic Turbidimetric Analysis of BET often shows inconsistent spike recovery of Endotoxin. For same sample and same Endotoxin standard dilution are shown different spike recovery. Sometimes the auditor asks me why this difference. These results did not indicate reproducible result. Can anyone share with this issue ?

A2: I do not think you should be surprised by the variations you see as long as they are within the inherent error of the test which is (+/-) one fold dilution (50-200 %). The reaction mixture contains multiple reactants the level of which we can not control in a consistent manner at the moment. In addition in KTA as well as Gel-Clot we are measuring an event that is well distanced by a cascade of reaction from the original interaction of endotoxin and Factor C amplifying the sensitivity of the test as well as its errors. Hopefully, the introduction of recombinant Factor C dependent tests in the future will sooth these "chemistry" minded auditors by minimizing the test error.

A3: I have a couple of questions for you to help clarify your issue. What is the range of the of spike recovery inconsistency are you seeing? Are the recoveries outside of 50-200% (which is the range of acceptable PPC (positive product control or "spike") recovery)? This range is considered to be the error of the test.

If you see a lot of variability in your sample PPCs, you want to consider more dilutions, out to your MVD. Inconsistent PPCs indicate that you are still working in a range of interference or need further method development.

A4: What do you mean by inconsistent recovery of the PPC? Do you mean you are getting instances of the PPC being out of range or the PPC recovery rates are not consistent (but within range).

There are lots of reasons a PPC may be out of range. Many of them relate to the product/materials. Some initial questions you might ask:
-Do you know the pH of the samples before mixing with the lysate?
-Does the next dilution in the series get proper PPC recovery all the time?
-Is each batch of product made from a new batch of raw material?

There are lots of reasons you might get inconsistent PPC recoveries (all within range). Many of them relate to assay performance. This type of variance is, generally, not caused by your product. Some questions you might ask:
-Are the onset times for a particular standard (eg, 0.05) consistently similar? If not, the dilutions used to make the curve are not consistent or they have been sitting around for too long.
-"Hot spiking" can sometimes produce inconsistent recoveries because tiny variances in the spike translate to large differences in final concentration.
-Loosing part of the PPC. This can happen with splashing when loading plates or delivering part of the spike to the side wall (both plates and tube readers).
-Improper issue of pipetters, eg, residue or bubbles within the tip, wrong tip for pipette size, bargain brand tips, etc.

This is not inclusive, needless to say.

A5: Here PPC means Spike recovery in Kinetic Turbidimetric Analysis. Spike recovery is within range but for same sample and same dilution and same concentration show different recovery .

Linearity, Regression, Slope, Y intercept, Standard Deviation, CV of sample and Standard -everything are within specification. When I started validation for a product for three batches. Same concentration of a sample spike recovery-PPC as below :
1. 99 percent for First batch
2. 146 percent for second batch
3. 112 percent for third batch
where Theoretical Spike value : 0.5 EU

My process definitely is validated by HPLC potency determination method.

Is it consistent result ? Can any auditor accept ?

How can I accept this result for Validation.

A6: This is a perfectly acceptable result. You can not ask the test to perform better. As mentioned earlier, recovery data that is 50% -200% (+/- one fold dilution) of the theoretical spike is with in the accepted "error" range of the testes. You should set your validation acceptance criteria to 50-200% recovery which is used for most application. Unless you are looking at some special application, narrowing that range to values that are beyond the test's capability will be a recipe for further frustrations. You should also remember that alerts limits are set with the inherent error of the test in mind.

A7: 1. These PPC recoveries are all good.
2. The difference between 99 and 112, for example, are insignificant.
3. You may expect small differences when testing different lots.
4. When you want to compare things or demonstrate reproducibility, first eliminate variables. Make sure that you are testing the same thing. Otherwise, there will be variability, but it comes from the experiment design, not the assay. In your case, use the same lot prepared the same way. If you do this, you'll find the PPCs to be a lot tighter. There will still be minor differences. There is no need to perform reproducibility tests unless you suspect a problem in the testing results. That's why you run controls and have parameters for test acceptability.

The auditor should accept the results you have.

Malassezia furfur

I have been looking to bring in the mold Malessezia furfur, better known as the dandruff mold, for some growth promotion studies. I have seen that Pityrosporum media is sometimes used, as well Dixon's agar. Could anyone chime in, and advise me where I can find the growth media for this organism. As well I see that it grows in the oil layers of the skin, but I don't see anything about it being a obligate anaerobe. I would appreciate any information on the incubation requirement for this mold, and the appropriate media for sustainable growth.

A1: I do not know of commercial sources for either media. We have media recipes for both available on our website, however most of our Malassezia strains tend to grow on Pityrosporum medium at 30.0°C:
ATCC medium: 1072 Pityrosporum medium
Malt extract agar...........60.0 g
Ox-bile (desiccated)........20.0 g
Tween 40....................10.0 g
Glycerol mono-oleate.........2.5 g
Distilled water..............1.0 L
Autoclave at 121C for 15 minutes.

ATCC Medium: 2693 mDixon (modified Dixon)
Malt Extract.................36g
Desiccated Oxbile.........20g
Tween 40...................10ml
Peptone.....................6.0g
Glycerol.....................2.0ml
Oleic Acid..................2.0ml
DI Water.....................1.0L
Adjust pH to 6 with HCl
Dispense into appropriate vessel and autoclave at 121 degrees for sterilization.
For agar medium add 15g/L of Agar

One comment,
If you are choosing to grow Malessezia furfur because because you want to use/grow the dandruff strain, you may want to choose ATCC® MYA-4612(tm) Malassezia globosa, type strain CBS 7966 instead. Due to the use of culture-based technologies, it was incorrectly assumed for years that dandruff and other skin conditions were caused by Malassezia furfur. Scientists at Proctor and Gamble successfully sequenced the complete genomes of both Malassezia globosa (ATCC® MYA-4612(tm)) and its close relative Malassezia restricta (ATCC® MYA-4611(tm)), because they are the most commonly isolated species from human scalp and are associated with skin disorders, including dandruff. They are both present on the dandruff scalp, but M. globosa is the most likely initiating organism due to its high ability to secrete lipases which are used to aid in its digestion. ATCC® MYA-4612(tm) Malassezia globosa, the type strain was isolated from the skin of a patient with
Pityriasis versicolor in 1990 in London. It is the most commonly found strain of M. globosa, and the sequence has been deposited in the GenBank database, accession no. AAYY00000000.

The media to grow ATCC® MYA-4612(tm) is Leeming & Notman agar Modified MLNA media (notice the olive oil!):
ATCC Medium: 2737 Leeming & Notman agar Modified MLNA media:
Bacteriological peptone.............................10g
Glucose...10g
Yeast extract..2 g
Ox bile, desiccated.................................. .8g
Glycerol... 10mL
Glycerol monostearate................................0.5g
Tween 60..5mL
Olive oil...20mL
Agar..15g

Deionized water......................................1 L
Medium is sterilized by autoclaving at 110C for 20 minutes. For liquid media, omit the agar.

However, I have heard that Malessezia furfur can be the easiest of the Malessezia species to grow.

MIC CALCULATION

I need the method to calculate the minimum inhibitory concentration of antibiotics as a part of antibiotics sensitivity test, and any available reference for this issue.

Compressed Air Sampling

When performing environmental monitoring of compressed air, what is the standard volume of compressed air that should be analyzed for microbial growth? Is it the same as active air sampling (cubic meter)?

A1: Is their any reference for the sampling quantity of the compressed gas ?

A2: There is specific guideline for compressed air monitoring which is ISO-8573.1. hope this will be useful for you. the sampling volume is 1 cubic meter according to same.

A3: Following is the reference guidance for compressed Air

"BS ISO - 8573-1:2001"
Please refer it

Residual VPHP (VHP) in Isolators

Let me introduce. I am the responsible of a Micro Lab (API manufacturing company). We manufacture sterile API and we use VPHP (VHP) to sterilize isolators. Following VHP dosification, an aeration phase is performed in order to remove VHP from the isolators. As the sterile API contacts with the environment of the sterilized isolator, our concern is about the maximum residual allowable limit for VHP within these equipments.

Incubation time for microbial examination of non sterile products

in Ph Eur 2.6.12. and 2.6.13. incubation time for tests (Microbial enumeration tests and Tests for specified micro-organisms) are given in a form of range (for example 3-5 days). Should the test result be released after the longest time of defined period of incubation has expired or the result can be released after the shortest time has expired (for example, should the test result be released after 5 days of incubation or could it be released after 3 days of incubation)?

Food Industry Microbiology lab layout & required equipments

I need to know what are the layout & facility design for a microbiology laboratory for QC of food industries' products. If there is any references for that please forward ASAP.

Reduced testing for commercially available media?

For those of you who use commercially prepared contact plates and TSA, have any of you justified reduced incoming testing based on previous reliability? That is, not performing growth promotion

verification on each lot, rather on a lower frequency (every 5 lots, or perhaps twice per calendar year as examples).

BSC hoods

I am currently looking add a 6foot Class II Type A2 Biological Safety Cabinet. I am familiar with Labconco, and have received quotes from them, but would like recommendations for other suppliers.

A1: If you are using the hood for cGMP use you might make sure that the framing on the HEPA filters is not made of wood. Wood is not allowed in BSCs for cGMP use. We found that a small percentage of our BSCs had the wood frames for the HEPA filters. The BSC technician said that mold may grow on the wood frame over time.

A1: Can you provide a reference stating that wood framed HEPA filters are not allowed in BSCs for cGMP use? My certification tech has never heard of it, nor seen any mold since the air-flow is so high.

A2: The reference in the EU orange guide appendix 1: in the Premises Line 22.
"In clean areas, all exposed surfaces should be smooth, impervious and unbroken in order to minimise the shedding or accumulation of particles or micro-organisms and to permit the repeated application of cleaning agents, and disinfectants where used." Plus the EU orange Guide say wood is not allowed in clean rooms (much like paper and styrofoam).

Production Line 7 "Materials likely to shed fibres or other contaminants, like cardboard or wooden pallets, should not enter the areas where products or clean containers are exposed."

It is stretching that premise of there should be no porous materials that would harbor organisms.
If you turn off the BSC the airflow would be off.
Some people do leave the their BSCs on all the time.
The BSC technician told me that he has seen some mold on the BSC filters. That is why they suggest that they are on 100% of the time.
They do make metal frames for the filters too.

When someone flagged the wood frames and being inappropriate I was like no big deal too.
A metal frame would be better.

A3: Look at
Terra Universal - www.terrauniversal.com (This is a gr8 source of products for other lab equipments too)
Thermo Electron - www.thermo.com
Esco - http://us.escoglobal.com/index.php
CleanAir - www.cleanairtechnology.com

A4: I think the important thing to remember is that this industry (and this list) is quite diverse and it is our own responsibility to take the available guidance and it apply it to our unique situation (product line, environment- sterile vs non-sterile, location, etc).

Rabbit pyrogenicity test

I am a microbiologist in the only drug-production company in Albania.

Can someone give me information about testing of pyrogenicity in the Rabbits? Do you use this test in your countries? if yes tell me your countries' names and the reason because I have heard that is a tendency to replace this test with LAL test. For each solution that is 10 ml or more than 10ml p.e.NaCL 0.9%-250ml, NaCL 0.9%-500ml, Glucose-500ml, Metronidasole -100ml I do the LAL test and the rabbit test too. It will be very helpful to me if anyone respond me and suggest or tell me what you do in your

company because I have to decide to use or not more the rabbit test for pyrogenicity of the solution that produce our company.

A1: A long time ago the rabbit test was replaced by LAL test this is 20 times more sensitive. Before to use it you have to validate. All our endotoxins tests are made by LAL method in our company. You can find information in USP.

Rappaport Media

I would like to know in what temperature do you sterilize Rappaport.

We tried to validate the sterilization process using 115ºC but we didn't reach the lethality rate.

Is it right to sterilize using 121ºC if I prove that I am able to promote the develop of Salmonella sp?

A1: Sterilize the Rappaport Broth at the manufacturer's recommended temperature of 116ºC, but increase the time from 15 minutes to 25-30 minutes. The longer time did not affect the quality of the nutrients. I also sterilized it at 121ºC to see if any alteration occurred in the quality of the nutrients, and I was able to grow Salmonella enterica very well.

A2: No, as per USP you are to sterilize this media only at 115 deg cel only.

A3: You should follow the supplier's instructions . The cycle should be validated so that the agar reaches 115ºC for 15 minutes not that you reach lethality. The autoclaving at 115ºC will destroy vegetative bacteria. The medium is designed so that other organisms are inhibited from growth. The levels of malachite green and magnesium chloride allows only Salmonella organisms to multiply freely.

A4: We have one autoclave only and it is set at 121 only... if we have to set it at 115 then its a very long procedure for maintenance department. so we are doing with other media at 121 for 15 min and getting the results in growth promotion of salmonella.

A5: I agree with you [name redacted]. We should follow the supplier's instructions for the reasons [name redacted] mentioned plus that supplier stability data on media ingredients. So, if someone try to increase the sterilization temperature, & no drastic effect occur (or appears as that), this is very erroneous attitude. keep in mind that a wide range of other media shouldn't sterilized at all, such as XLD. I used to sterilize RVS media at 115ºC for 15 minutes even before USP harmonized chapter published, because these instructions are written at FDA BAM (a long time ago).

http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm063568.htm also the following statement is written: This medium must be made from its individual ingredients. Use of commercially available dehydrated media is not recommended.

A6: You can achieve the lethality rate by using the Biological indicator having D value related to 115 degree celsius. secondly you can not autoclave the rappaport medium to 121 degrees because it denatures the property of chemicals which support the growth.

A7: You can purchase this media ready made. We are purchasing it for a number of reasons, one it is cheaper to purchase than to make due to the quantities we use, plus it means the autoclave is not running a cycle for a few bottles.

A8: We currently autoclave RVS Broth, Merck, product number 1.07700.0500 (10ml in test tubes) at 121C for 20 minutes and have no problems recovering salmonella. Can someone confirm that this media should not be autoclaved at this temperature/time and why?

A9: USP says not more than 115C

Dissolve, warming slightly. Sterilize in an autoclave using a validated cycle, at a temperature not exceeding 115. The pH is to be 5.2 ± 0.2 at 25 after heating and autoclaving.

A10: Hi Eoin. This is from the Merck Manual: "...autoclave gently (15 min at 115C)." It's great that you're getting growth with the higher autoclave conditions. The 15 min at 115C was designed to optimize growth and there are some cases where a user may not have success growing or cultivating their specific strains of Salmonella for a variety of reasons if they use the harsher autoclave conditions.

A11: We use the same medium as you.
The following are the preparation instructions of the medium:

"Suspend 41.8 g/litre, heat gently, if necessary dispense into test tubes,
autoclave gently (15 min at 115 °C).
pH: 5.2 ± 0.2 at 25 °C.
The broth is clear and dark-blue.
The prepared culture medium can be stored in the refrigerator for at least 7 months (VASSILIADIS et al. 1985)."

We can't modify the autoclaving conditions, so we heat the tubes in a water bath at 99º for 15 minutes. We never had contamination problems.

Sterility testing

Once the sterility method has been validated for a product & GPT of the prepared media is done before its usage, should the positive controls include all USP indicators over and above in-house environmental isolates or only inhouse environmental isolates are ok for routine sterility testing?

A1: I do not understand how and why you should include organisms in routine sterility testing? Where do EP/USP describe positive controls, apart form GPT testing on sterility media?

A2: If you perform GPT of every lot of sterility test Media with required cultures including Environmental isolate, there is no need to run positive control.

A3: Positive controls have not been performed for routine sterility testing. Only negative control has been conducted. The use of microorganisms in parallel with the sample test is a risky practice (false positives could occur).

A4: Sterility test positive control is not performed in the same area where sterility is done, in fact it is done in the controlled area meant for handling organisms.

A5: If you growth promoted media using EM isolates and the regular USP recommended organisms in April and if this media was used in May for a sterility test (because it is still within its expiration dating)-How would you know in May that the media you are using still supports growth and will grow any contaminants ? In a nutshell ,how would you know if your sterility test did not give a false negative result ?
Does anyone perform a Last-use or post expiration growth promotion on a media batch that has already been growth promoted and released.This is to show that the media was sterile (negative controls) and was able to support growth (positive test plates) throughout its shelf life.
Just a thought..
Experts in growth Promotion- Please advise.

A6: You can verify shelf life of your in-house prepared media by performing GPT at different intervals for at least three lots of media prepared on different days. Once you have validated media shelf life, you can discontinue your extra GPT, which you perform to prove that your media is supporting the growth on the day of sterility testing.

A7: The test to which you refer is called the Stasis Test and is a requirement of my (Australian) regulatory body at least. The most practical application is to inoculate actual sterility test vials at some point soon after the 14 day sterility test has been completed...all the compendial bugs, plus a few local EM isolates etc.

Particulate Matter testing

I have a question about particulate matter testing. I was told analyze elastomeric closures for particulate matter. Does any one know how this test should be run and were I can find it in the USP.

A1: You will not find it in USP. However, ISO has the following document that specifically discusses how to analyze closures:

ISO 8871-3:2003

Elastomeric parts for parenterals and for devices for pharmaceutical use -- Part 3: Determination of released-particle count

Just so you know, this document "does not specify particle contamination limits. These will have to be agreed upon between manufacturer and user."

A2: I am not sure if closures are tested for particulate matter, but you can rinse many of them (10 or more) in 20mL at least of WFI, then check their particulate matter using pure WFI as control. May be chapter <788> in USP is about particulate matter.

Reaction Tube for BET, Gel Clot Method

I used to depyrogenate reaction tube for my BET test. I'd like to know if washing the tubes with water or detergent will change the surface chemistry of the tube because tubes that I received recently appear dirty and oily.

My supplier told me not to wash it for that reason. Anyone have idea whether the statement is correct?

A1: This is a very good question and I do know from experience that washing soda lime reaction tubes does change the surface characteristics that appear to be helpful in the formulation of the gel clot. This reduces the assay sensitivity and may interfere with your ability to achieve label claim.

I am not sure if rinsing borosilicate tubes would affect the assay for chromogenic or turbidimetric LAL.

A2: Rinsing of tubes with water and detergents does not change tube surface chemistry, provided that you have glass reaction tube. Confirm whether your tubes are of Borosilicate only and must have supplied with authentic COA. Generally tube becomes dirty if not cleaned properly and subsequently depyrogenated. So care shall be taken for proper cleaning so there is no residue of lysate/ CSE remains, before depyrogenation.

A3: I will suggest you to use disposable tubes for PET it just cost 2 rupees per tube. Washing with any kind of detergent does not completely remove the endotoxin. and no oven does complete depyrogenation.

A4: i haven't came across any such interference due to washing. We use to wash up tubes thoroughly with boiling water, just keeping in mind that gel content should be removed completely. Then we depyrogenate it before taking it in use for the test.

A5: There are two kinds of glass test tubes for the Gel-clot technique, borosilicate and flint.

The most problematic issue of glass on BET is leaching alkaline from flint glass. Borosilicate glass does not usually release significant alkaline, but flint glass releases alkaline in the reaction mixture to change its pH. If the LAL reagent used contains enough buffer components, the test results will be no problem. However, some of the commercially available LAL reagents do not contain enough buffer components. When you use such kind of LAL reagents, you must use the test tubes recommended by the manufacturer. If you use different test tubes, you may have unexpected results. I think washing does not make anything on the surface chemistry of glass, but washing of flint glass decreases leaching alkaline in the reaction mixture. It may cause significant pH change in the reaction mixture.

In your case, if you purchase depyrogenated test tubes from your LAL manufacturer, I think you had better use the test tubes as they are because the manufacturer established the labeled sensitivity of the LAL reagent with their test tubes. If you observe inconsistency of the results, you should report it to the LAL manufacture and ask them to improve it.

If you prepare depyrogenated test tubes and you find dirty and oily test tubes, I think you had better wash them because dirty and oily test tubes may give you inconsistent results. You need to confirm your LAL supplier's recommendation.

A6: If the tubes are coming from an LAL vendor, then the appearance could be caused by the tubes being packed too tightly and/or cooked too long in depyrogenation. The tubes should work OK.

However, your comment makes it sound as if you are buying bulk tubes. It is not normal to see oil on tubes. Could this part of the appearance be caused by something else? As to dirt, bulk tubes are frequently dirty. Two common causes are packaging lint (paper fibers) and a powder applied deliberately to control humidity. The packaging lint will burn off in depyrogenation if your cycle is hot enough and long enough. The powder must be washed out.

If you are using a detergent (as opposed to hot WFI/PW), then the choice of detergent is important. Milder detergents will leave a residue on glass, which can adversely affect lots of types of assays.

The type of glass can also affect the performance of the gel-clot assay. Be sure to use the type of glass recommended by your LAL vendor. There are differences in the formulations, and it shows up in the type of glass they recommend for best performance.

A7: In my opinion also the tubes should be washed properly so that no residue is left behind which will cause problem to the reaction.

A8: Thank you for your reply. Actually I'm buying soda lime tubes in bulk & depyrogenate in-house. Tube is discard after each use.

pH adjustments

We have a lot of raw materials that are regularly tested for microbiological purity. 10 g are weighed, and 90 ml of buffered sodium chloride peptone solution are added (1:10 sample solution). Ph. Eur. says that if necessary, pH of the solution has to be adjusted to 6-8. And there's the problem.

Some of the solutions have an impossible pH. For example, tartaric acid 1:10 solution has a pH of 1.82. When 20 ml of the 1 M NaOH are added (10 g + 70 ml diluent + 20 ml NaOH), pH rises up to 2.49, when 50 ml of 1 M NaOH are added pH rises up to 3.15. Possible solutions to my problem could be:
- to use NaOH that is more concentrated (2 M NaOH or higher?)
- not to perform microbiological tests on tartaric acid based on a risk analysis (non-friendly environment for microorganisms - low aw, low pH...)

What do you think?
How do you adjust pH for your solutions? What concentrations do you use (NaOH, HCl?)?
Do you perform microbiological analysis on materials like tartaric acid?

A1: Use much more concentrated NaOH, for example 10N. Perform a titration against your product in the buffer solution. Then for routine testing add the required amount of NaOH to the buffer solution before adding the 10g of product. You should do a few titrations to make sure that you get it right. And good luck with the validation.

A2: If you know how strong your tartaric acid is, you can calculate how much (m)mol NaOH you need to add for a pH between 6-8. If you know how much (m)mol NaOH you need to neutralize tartaric acid, you can calculate how much and how strong your NaOH need to be.

If you use a strong NaOH solution, please be careful with adding it. Micro-organisms don't like it.

A3: This problem will be solved by validation but you can try 10g + 90 ml 1N NaOH, prepared from sterile DI water in aseptic technique.(Similar to that mentioned in preparation of LAL NaOH but with WFI water). However, can bacteria present in ph 1.82 grow in TSA with ph 7.3. Validation is important to check if TSA is suitable or you need to use another media.

A4: In the case of tartaric acid, we use 5N (or higher) NaOH and buffered sodium chloride peptone solution.
But, Ph.EU says that if necessary, pH of the solution has to be adjusted to 6-8, so I don't think you have to adjust pH.

I think there are no normal microbes in tartaric acid!

A4: Use membrane filtration method. Tartaric acid is very soluble in water (~133 g in 100 mL). Harmonized method (USP/Ph.EU) allow membrane filtration method.

Tartaric acid has such a lovely well balanced structure, I remember this from my college days (always used as an example of Levo and Dextro rotation).

decontamination of used media

Decontamination of used media using PHENOL disinfectant, instead of PHENOL any other is any good Disinfectant.

A1: BETTER TO DECONTAMINATE BY AUTOCLAVING IN BIOHAZARD BAGS FOR 30 MINUTES & THEN DISCARD IN EFFLUENT TREATMENT PLANT.

A2: I have seen 10% bleach used in China, but generally would recommend decontamination by autoclaving all contaminated items.

A3: When you are performing the decontamination by sterilizing the used media in a validated cycle, what is the need for phenol?

A4: Please, what do you mean by decontamination of used media using Phenol disinfectant explain more and for what you use this media.

A5: I have seen labs in Asia that do not have autoclaves available, and they soak the contaminated plates and other contaminated products such as pipetters in some disinfecting solution before discarding them in regular trash. Not a good practice, but it is being done in the absence of autoclaves.

A6: Decontamination by two methods 1)Sterilization and 2)Disinfectant.In my point of view better to decontaminate your media by validated sterilization(steam sterilization) method.If you would like to decontaminate your media by disinfectant method you need to select suitable disinfectant and go for validation.My choice is 0.5% sodium hypochlorite for minimum 1 hr contact period.Check the details of this disinfectant and go for further.

probiotics

I am having an issue with a product that has a high total plate count that exceeds the TPC limit for complimentary medicine as set by the T.G.A. The product is a powdered dietary supplement that contains lactobacillus as well as Bifidobactera During our last audit by the T.G.A. we were cited because we released a product that had a total plate count result higher then the T.G.A. limit. Our justification for releasing the product was that it contained a live culture, and that the live culture was responsible for the high TPC result. The T.G.A. does not accept this, and I was hoping to get some advice on how we can show that when the lactobacillus count is high, the total plate count will be high as well. In addition to the TPC test we also test every lot of this product for enterobacteria, coliforms, e coli, salmonella, yeast, mold, & staph. Below is the comment from the T.G.A. any suggestions would be greatly appreciated.

[name redacted]

"The product exceeded finished product total plate count specification but was released on the basis that the product contained Lactobacillus as an input material and the input Lactobacillus caused the high plate count results. While there were test results that showed high levels of Lactobacillus and Bifidobacteria in the finished product, there was no link or test results to demonstrate that the high plate counts detected were due to these two organisms. Additionally, the recommended removal of the total plate count limit from the finished product specification may be premature as it was based on one batch of product with insufficient supporting data."

A1: On what media do you perform your TPC? Bifidobacterium does not grow on aerobic incubated TSA and SDA plates and Lactobacillus will hardly grow out. I qualified a TPC for oral finished product containing live freeze-dried Bifidobacterium and live freeze-dried Lactobacillus. All spikes were picked up really easy! FDA accepted.

A1: Usually the Probiotic bulk TPC limits other than the Probiotics is NMT 1000 cfu. When you plate for TPC with Plate count agar or SCDA , Lactobacilli and Bifidobacteria do not grow and only the contaminants grow.

I think your justification for more TPC may not be correct.

A2: lactobacillus as well as Bifidobactera are anaerobes should produce high count in anaerobic culture which usually based on using MRS or equivalent media. total plate count is for aerobic & facultative anaerobic species which usually based on using TSA or equivalent media. Finally, did you identify the bacterial species which cause this high total plate count (if it means total viable count), because there may be a true contamination problem. For probiotic product COA you should distinguish between the results of microbial limit test i.e. contamination, & the results of total lactobacilli or Bifidobacteria count i.e. assay of active principle.

A3: The specifications of finished product containing probiotics are as follows:

1)Total viable count: 80%-300% of label claim

2)Bile-tolerant gm -ve bacteria: < 1 X 102 cfu/g or ml

3)TMYC: < 1 X 104 cfu/g or ml

4)Pathogens:

*Escherichia coli: Not detectable per g or ml

*Staphylococcus aureus: Not detectable per g or ml

*Salmonella spp.: Not detectable per 10 g or ml

So if You use these specs then your problem will be that TVC exceeds the 300% of label claim of your product.... if that right you shouldn't have release this product because it's beyond the limit.

But if the count is very high but not exceeding the 300% of label claim so you need to prove that most of this count is due to your input strains using morphological shape on agar, gram stain and biochemical tests even API strips.

A4: Hi [name redacted]

Can you please provide a reference source for your spec; specifically the 80 - 300% of label claim bit?

We continually dance around different interpretations of the harmonized compendial counting interpretation (<100 =200, <1000 = 2000 etc) and have loosely called this a 0.2 log factor or even +/-20%. Previously, of course this would have been 0.5 log or +/-50%.
These figures are okay when dealing with relatively small numbers such as found in contamination testing, but start to get a bit silly when your lower limit spec is 300 billion (+/- what exactly...?) I'd be a bit worried if I needed to apply a +300% acceptance to that number...this stuff is really expensive... and if that +300% is attributed to non-lactic contamination then we're really in trouble!

Hi [name redacted]

We too are regulated by the TGA and have had few issues, especially if you correspond directly with TGAL; they are well informed and pragmatic. Interpreting the TGA comments you supplied, I would think that the issues they have are that you have no clearly defined way of segregating probiotics from contaminating TVAC, and that you apparently ceased performing TVAC test without sufficient justification. Have you looked into other alternate media for TVAC (for example, the dairy industry uses Nitrate agar to provide for some, at least, contaminants to show without the aerobic probiotics getting a look in; another option seems to be Sugar Free agar for the same reason). Neither of these agars is ideal however, and I am intrigued to see the comment tucked away in the harmonized compendia that TVAC methods suggested are not appropriate for use when the active ingredient is a live culture...seems to me that the compendia have just realized that probiotics are in the marketplace but they've no idea how to test them...it's up to (and me) to justify our standpoint to the regulators, and possibly to use some Risk Assessment procedure to formally explain your position; after all, what really is the impact of a thousand benign bugs among 300 billion "good guys"...?
(pathogens excepted, of course!)

A5: Probiotics such as Lactobacillus acidophilus are capable of growing under aerobic conditions. We have seen recoveries of upwards of 40% input (1 x 10^12 cfu/g) under aerobic conditions (TPC isolate identifications confirmed hypothesis). This becomes a major problem for finished product release. The ability for the L. acidophilus to grow tends to vary with the raw material batch, perhaps due to the time in relation to material processing or some other mechanism underlying lot to lot variability. Either way, if you need to perform a 1:10^8 or 10^9 dilution in order to get reportable results, you are not going to see any sort of contaminants (or at least the probability is extremely low) that may be present anyway (unless something went horribly wrong in your process). In this situation, it is in your best interest to remove your TPC specification and rely on your raw material results, your environmental monitoring, and the water activity of your product to justify removing the specification.

probiotic validation

Our company has a a probitic powdered containing aprox. a mixture of 4,5 x 109 CFU of Bifidobacterium infantis, bifidobacterium bifidum 1,50 x 109 CFU and bifidobacterium infantis 1,50 x 109 CFU we need to validate the analitycal method for this product. We perform an acid latic bacteria total count on MRS agar (CO_2 atmosphere) (intern method). I have the following questions:

1. How could we validate this method, when a microbiological validation require adding a known bacterial inocula size and this product is already inoculated? or we must only validate count method performance?

or should we elaborate a placebo without acid lactic bacteria and then adjust a inoculum size for each one according we find in the product and finally adding each inocula to the product like we perform in non-sterile product validation?
2. Is it correct performing a total count of acid lactic bacteria when the product has different kinds of bacteria?
3. Which standard, guide, regulation or USP chapter talks about probiotics validation or analysis?
4. Which are the validation criteria for this kind of products?
4. How can I validate the S.aureus, P.aeruginosa. Salmonella, E.coli investigation and aerobic and mold and yeast total count?We perform this analysis according USP chapter 61 and 62. I think I can validate this microorganism group like for non -sterile products. Is this correct?

A1: I have been reticent to dive in here because I've only been working with probiotics for less than a year; I'm NOT an Expert! However, some of these questions do seem to have fairly straight forward responses, so here goes.

1&2: Validate Total Count method only, and this is really just a question of comparisons with other data sets; we compare to CofA and previous tests; it's virtually impossible to truly "validate" a total count method; what you get is what you get. You could ask a consulting lab to run parallel for a given time/set of samples, defined in a protocol. Therein, also define your acceptance criteria.

If you are counting a deliberately mixed culture, then your Total Count will be just that. It may be enough if the label claim says it's an undefined mix. If there are numbers on the claim for each species, you'll need methods able to support. We use oxygen as a selective agent; the Bif won't grow so an aerobic incubation will give you just the lactobacillus component. If all are bifs, you're out of luck using oxygen (unless one is actually facultative ...see my ignorance creeping in there?)

We also use morphology in a three way blend, but I'd guess with 3 bifs that won't work either? Rates of growth? How about antibiotics? There are dozens that are really specific to genus, and often species...again, with those 3 bifs you might have some problems. No real answers for you, perhaps a couple of directions? Sorry!

3: Compendia have only just got their heads around the concept of regulated probiotics. All I can find is that they now acknowledge that TVAC methods are probably not going to work with products containing live cultures as an active. In order to prove absence of contamination (<10,000cfu/gm), firstly, declare them to be non-lactic contaminants in your spec. Then try to find a way to knock off those probies...aerobic TVAC would seem to be fine if all your probies are obligate anaerobes? No problemo! Otherwise, try Sugar Free agar or Nitrate agar as starting points; it may be that you require further refinement of your Non-lactic spec definition.

4: Yes, correct; treat them as non-steriles; obviously they are anyway, but that's the only section you should have been looking at in the first place. Most of the specific bugs will actually validate but you might find that the outgrowth of the probiotics lowers the pH; try using Buffered peptone Water instead of TSB (that's why it's buffered!!!); if that doesn't work try actually shifting the pH back up an hour or three into the enrichment; this is what the food industry has been doing forever. Salmonella is the only one giving me grief at the mo'; all the others came good first time around.

petrifilm for environmental monitoring

During a recent audit we received the following comment:

"The company used petrifilm instead of the traditional air sampling method for microbiological monitoring. There was no validation carried out to determine that petrifilm was suitable for the intended purpose. Additionally there was no quality control test or verification of each batch of petrifilm received to demonstrate the suitability of the batch"

I'm not real sure how to address this as I was under the impression that petrifilm was fairly accepted and know for use as a method to monitor air. We are a non sterile nutritional company and use the y&m and aerobic plate count petrifilms weekly throughout our facility. One reason we use the petrifilm is that it

seems to be less likely to be compromised through user error then traditional open plate methods, in regards to potential mishaps in preparing the media. I am not sure how I would go about validating the petrifilm for environmental use. Should I simply use petrifilm along side of traditional TPC plates and show that they correspond?

A1: You need to "validate" petri film to show it is equivalent to the media that are used in the industry (e.g. TSA with neutralizers). The validation should consist of at least three series of side-by-side comparisons using a battery of typical growth promotion organisms and some of your environmental organisms. Once validated you will need to qualify each batch upon receipt by conducting reduced growth promotion testing - this should mimic the testing you do (or should be doing) for each traditional batch of media you purchase or prepare in house.

I know that no one likes to do GP because it is a pain in the, um, neck, but it's probably the most important test a lab needs to do because everything is contingent on the media supporting the growth of the organisms you are propagating or attempting to isolate. It's one of the necessities of running a competent laboratory.

A2: I don't think you were commented on because you used a different method of air sampling. You probably received this comment since you did not have documentation to show that your new method/ equipment was comparable to the traditional method/equipment. You need to perform some sort of a comparability study with an intent to show that whatever you may have recovered using the traditional method or equipment, you will get similar or comparable recoveries if you were to use the new equipment/ method. You would have to customize your own study.Take into account how many facilities you have,the common EM isolates in each facility , historical EM trends. You would also need to perform a risk analysis to show that the new method or equipment does not impact your product performance or impact the form, fit and function of your product. Please include your most common EM isolates as the challenge organisms in this study.

Evaluate bioburden on PPE Lab coats and gloves

I have been asked to perform a study to evaluate the bioburden on lab coats and gloved on personnel in our manufacturing areas. We are a non-sterile manufacturer. Can you suggest the most effective and the most valuable way to go about this testing? How is this testing performed? Should testing be performed on all manufacturing personnel or a select number? Is there a reference that should be followed? Should all isolates be identified?

A1: Nonsterile area contains class C (1000) & D (100000). Class C should be tested twice a week (including personnel) while D which mostly is Packaging area once a month. Go back to USP < 1116>

Dilutions issue

I have a product and I would like to initiate a microbial test method for it. The limit of the TYMC is no more than 10 cfu/g and NMT 100 cfu/g for TAMC. I performed a method development using 1:6 dilution in pre-warmed TTL. The out come is viscous and I couldn't even vortexed or shook it. The product is similar to xanthum gum.
My question is what kind of dilution or alternative solution I should use to meet my limit of NMT 10 cfu/g.

A1: Two things i'd like to understand: Who decides the limits. Home limit or from official Pharmacopea. Second try 1:10 dilution as written in USP & EP.

A2: If you use 1/100, use ten plates instead of one, or use larger plates(20mm or larger).

A3: Why 10 plates?
Because you test the same amount of product if you do the test with a 1:10 dilution?

That's not the right interpretation of your result.
If you use a pour plate method with a 1:10 dilution, your limit of detection is always <10 cfu/g. You test 0,1 gram and 1 colony is 10 cfu/g.

If you do the same test with a 1:100 dilution, your limit of detection is always <100 cfu/g. You test 0,01 gram and 1 colony is 100 cfu/g.

If you test 10 plates with a 1:100 dilution, every plate has a limit of detection <100 cfu/g. If you find nothing, your result is <100 cfu/g.
If you found 0,1,0,1,0,1,0,1,0,1 ,
your arithmetic mean is 0,5 *100= 50 CFU/g, BUT this result is lower than your limit of detection, so you have to report <100 CFU/g.

A citation of the Ph.Eur 2.6.12, 5-2-2 plate-count methods, 5-2-2-1 pour-plate method:
'take the arithmetic mean per culture medium of the counts and calculate the number of CFU per gram or per milliliter of product'.

I have no experience with your product. For a filtration method you can use a greater sample dilution, but you have to filtrate a lager amount to filtrate 1 gram or 1 ml of your product. If you use for example a 1:100 dilution, you have to filtrate 100 ml.

Positive Controls for the Growth Promotion Test

According to USP 32 Chapter 61, when performing the growth promotion test on a new media, the test media is qualified if: " For a freshly prepared inoculum, growth/clearly visible growth of the microorganisms comparable to that previously obtained with a previously tested and approved batch of medium occurs”.

When performing the Growth Promotion Test on a solid media, can we just compare colony numbers from the test media to numbers obtained on a previously qualified media tested on a different day? Do we have to have positive control plates each time we do a growth promotion test on a new solid media, or can we compare CFU counts from the new media batch to CFU counts obtained from an approved media batch on a different day? Similarly for liquid media, do we have to have a liquid positive control media each time we perform the test, or can we just have a control for colony counts to make sure counts delivered to the liquid media were between 10-100 CFU?

Input and discussion on how others are interpreting chapter <61> and how they approach the use of positive controls in growth promotion tests will be very helpful.

A1: We compare the count of the new medium vs. the count of the previous approved medium; but both counts are performed in parallel.
This procedure has an additional advantage: we assure that the previous approved medium has been meeting acceptance criterion along its used period.
According to our experience, the counts performed in different times, in general, cannot be compared; so the counts of the new and the previous batches have to be performed in parallel.

For liquid medium, a positive control is not necessary. We assure the inoculum with 10-100 CFU by the count in triplicate in solid medium.

Training videos

Can anyone recommend any lab safety videos that could be used as training tools for new hires. Specifically I would be interested in microbiology lab safety and GLP orientated materials.

A1: I've found many video trainings on Youtube, I think the best one is from RMIT. Use keywords such as: microbiology safety training, etc.

UV challenge

Regarding challenge test for UV pass box what is the acceptance criteria for the UV intensity test that is performed before the bacterial challenge test?
Can any one send me any guidance regarding this test?

A1: We challenged the UV pass box with microorganisms, and we pre-established a reduction of more than 3 logs as acceptance criterion.
Microorganisms were spread on TSA in plates, and different exposition time periods were evaluated.

A2: The following approach will help you:

1. Determine the bioburden of items to be transported through the UV pass box (Why? To know what is the type of bugs to be challenged & the inoculum size to test)
2. 2. Determine the load configuration for the items.
3. 3. UV bactericidal effect is time dependent. Therefore based on 1 & 2

inoculate the items with not more twice of count of the bioburden and challenge them on different time intervals.
Regarding to UV intensity test this is a physical test can be determined using calibrated UV intensity meter.

A3: We validated decontaminating Pass box by using 106 of Bacillus subtilis. We placed one ml of the culture on opened Plastic Petri dish (disposable)for many periods then we added directly TSA media after the specific period under safety LAF. Till we got 6 log reduction.

We opened the plates because when we tested the transmission of the UV (The killing Wavelength) through the plastic cover in separated experiment but it didn't pass.

We didn't use pre poured media.

Be careful, check firstly if the UV rays penetrate from the plastic or glass cover of the UV tube. We removed it because we found it blocked UV.

Question

I am new to the pharmaceutical industry have dealt with the food industry. That said I have never dealt with a cleaning room before. I am reviewing an already made S.O.P. for the Environmental Monitoring Program. It states that the plates (RODAC and settling plates both containing Tryptic Soy Agar) first be incubated at 20-25°C for 72 to 96 hours at that time they are checked and then receive additional incubation at 30-35°C for 48 to 72 hours. The goal is to count how much bacteria there is but at the same time check for "objectionable organisms" such as Salmonella, Escherichia coli, Pseudomonas, Staphylococcus, and Candida. What I was wondering is all those except for the Candida albicans are usually incubated near 35°C will incubating them at the lower temperature first effect the growth if there is fact these bacteria present. On the other hand the clean room will be around room temp, so it might be more representative to have the incubation at the 20-25°C to simulate the environment in the clean room. Any info would be helpful.

A1: I assume your BOP is set up to utilize one media to detect both Yeast and molds as well as vegetative bacteria on one general growth media (TSA). I have seen some sites with a similar program that do this versus plating two plates or two different kinds of media at two different temperatures. The idea is to incubate at the lower temperature first to detect yeast or molds and then at the higher temperature to detect bacteria so that the bacteria do not overtake the yeast/mold before they can be detected. In my opinion, to detect yeast/molds it would be preferable to use media formulated for molds such as Sabouraud dextrose that has a different pH and incubate them at 20-25 degrees so that results can be provided sooner by incubating simultaneously as the TSA plates.

A2: Someone designed your program and decided on the parameters. It is not a standard from anywhere meaning that you can change it. With that said, there would need to be a formal justification for the change and you brought up some good points. However, your products have been manufactured with the existing standards so you will need to show that any modifications result in something equal to or better than what you are changing from. If you can find no justification for the current program than you have a problem that needs to be fixed. Objectionable organisms in particular need to be justified. The criteria for what would be objectionable in a product can be found in USP <1111>.

Help needed - identification of contaminant

Can anyone help to identify this? It was found contaminating a flask of our mammalian cell culture. It grows as an spreading, orange colony on TSA, and is catalase positive. Any clues would be helpful.

A1: Yeast, maybe? Looks like some budding going on.

A2: Maybe a yeast-like fungus - check out genera Bullera, Sporobolomyes, Trigonopsis and Sterigmatomyces? See anything on lid of dish if placed in light?

A3: The Catalase test is not that useful of a test. It only tell you that it is not a lactic acid bacteria. Lactic acid bacteria are usually slow growers and would not be a primary contaminate concern. What you need to do is:
1) Streak the organism to purity on TSA plates.
2) Gram stain a colony.
3) Look at the cellular morphology under the microscope.

Then decide from there. If it is G+ large cells with or without budding it could be yeast.
Orange colored colonies are not that common. That would help in the ID.
Yeast many times are colored pink or orange.
Yeast are definitely larger.
ID you had SDA medium you may use that to see if it will grow on that. If it does it may be yeast.

A4: Hi Serena,

Those information you gave are not enough to determine the ID of your TSA growth.
First of prior, you may observe what is the characterization of morphology of the micro-organism under microscope. From a fresh growth perform a Gram stain to distinguish between: Gram positive or negative, rod or coccus or may be a yeast.

if the bacteria has a rod-shaped a fresh microscopic observation will help to determine the motility +/-. Or, on a tube motility . Know that bacteria are less motile in old culture.

From these steps, you can orient your ID to determine the species, of course If the growth is a bacteria, by using the adequate API-Bacterial identification strip (bioMérieux). A Vitek will be the best.

A5: I forgot others important details. After determining the Gram+/- and and the bacteria-shape and before performing the API - bacterial ID strip, an oxidase test should be considered according to the Gram-stains and the bacteria shape if the bacteria has a rod-shape. Or a diploccic-shape --some diploccique bacteria like Neisseria are oxidase positive

1/- Enteriobacteriaceae are Gram negative & oxidase negative (there are some exceptions)

- Pseudomonas are Gram - and typically oxidase positive (but according to your colonies discription dont think its Pseudomomas bacteria)

- Some others Gram negative stains are oxidase + like enteric bacteria.

2/ A catalase test is necessary if the cell-bacteria has a coccus-shape under microscope and are Gram-positive in coccus chains or Gram positive in coccus clumps. Always under microscope, the shape may help you to know the cocci family or its genius.

Bacelli, coccus and Gram+/-, oxidase +/-, catalase+/- will help you to choose the selective media. Then to choose the API-Bacterial ID strip or Vitek to know the species.

Serena, please note that the microscopic observations and those easy simple tests : oxidase and catalase are very significant to orient your ID to the genius or species level, to save your time, media growth and other consumables. They should be done prior to any ID but unfortunately many microbiology laboratory neglect them.

A6: Looks like a yeast? Maybe use an ID 32 C strip from Biomerieux to identify it?

A7: Don't know from the photo but you might consider Trichosporon sp. if it's yeast-like.

A8: Good picture. I think it must be yeast. Looks like ovals with hyphae or pseudohyphae.
Can you mix in a known bacteria for size? Yeast should be a bit larger than bacteria.
Can you plate it in SDA media or SDA with antibiotics. If it grows you know it is a Eukaryotic cell most likely yeast. When you grew it on a plate does it grow like a chocolate chip or umbonate?
Many yeast grow like that on solid surfaces.

A9: It looks more like bacterial cells than yeast to me. More like Flavobacterium sp, which are catalase positive, and typically yellow/orange colonies. Here is some description from internet:

TAXONOMIC DESCRIPTION

Flavobacterium are found in soil, water, plants, food stuffs and almost every water systems including distilled water lines and dental chair spray units. They can also survive in intravenous anesthetics, eyes, urine, and stool samples. Most species are yellow pigmented rods. Motility is not observed in hanging drop. Some strains form spreading colonies. Some species are acid producing. Flavobacterium are nonfermentative gram-negative bacilli. All but one species produces indole which is a unique characteristics among non fermenters. They are chemoorganotrophs which have respiratory metabolism. Some strains are holotolerant. Flavobacterium are fastidious microbes and some strains are pathogenic to humans and animals.

You should be able to easily identify through standard biochemical methods.

A10: Have you considered something like Moraxella sp? It is supposed to be Gram negative, but if I recall - may be mixed upon Gram stain (somewhat difficult to decolorize). It is also catalase positive if I recall (sorry - retired and don't have access to all the reference materials).

Environmental monitoring standards for nonsterile products

Are there any definitive/proposed environmental microbiological standards/guidelines for nonsterile solid oral dosage manufacturing areas ?

Would "at rest" monitoring be acceptable? What would be an acceptable frequency of monitoring by settle plates/contact plates/air sampler for the total viable count and pathogens?

A1: For nonsterile solid oral manufacturing areas mostly their class is C (10000) at rest & in the same time it is difficult to monitor them during dynamic work due to presence of powder which may give negative false, I prefer to test them just after finish or stop work, twice a week with action limits: 20 cfu/m3 for volumetric air sampling, 5 CFU/ contact plate for surface except floor & 10 CFU / contact plate for floor. (USP limits).

If you test the area before starting work you are actually checking the efficiency of the cleaning & this is another issue.

A2: There are no set guideline for EM in non-sterile facilities. Regulations are working on it, however nothing had been published yet.

A lot of people rely on chapter <1116> and using limits of class D (100000), not class C as [name redacted] recommended, since most companies don't have regulated air exchanges in each room. Other people collect data for a year or two and set internal limits based on the results collected.

Sampling schedules are vary as well. Some companies collect data once a quarter some other once a month (mostly based on number of rooms that need to be monitor).

Acceptance Levels

How does one establish acceptance levels for packaging , as part of the in-process control.

The $\sqrt{(n+1)}$ Formula being used.

Incubation time for Antibiotic Microbial Assay

I want to know in other organization how to apply the incubation period stated on USP Ch. 81 for most methods that stated 24 hours incubation time (i.e. Erythromycin - M. luteus) ? Do you strictly incubate for 24 hrs because I think it's not effective if at one time you finish working at 6PM (having an overtime) and need to see the result on the next day at 6PM.

I would appreciate if you can share the implementation at your organization.

A1: We usually have internal procedures (SOPs) that allow us to read plates/ results anytime after the end of incubation (24 hrs), not a minute before that.

If a test was performed and plates incubated at 6 pm on Monday, 24 hrs of incubation would end on Tuesday at 6 pm, per our procedure we would read these results anytime after 6 pm on Tuesday or on Wed morning (anytime before 6 pm on Wed).

This way my team doesn't have to do a lot of overtime and plates can be read once on Sunday morning for any tests that come off test on Saturday night and Sunday morning and we are still reading our results between the 24-48 hr time frame . Any organism growing on the plate at 7 pm on Tuesday will still be seen and recorded on Wed (obviously !!!).

You run a risk of non-compliance only if you read your results earlier than the 24 hr incubation time, anytime after that is still justifiable since any orgs present at the 23 rd hr will not vanish after 28 hrs .
but if you read your results at the 23 hr you can be questioned on the integrity of your results and asked how can you be so sure that nothing would grow in the next 1 hr.

Although more is not the answer every time, this seems like the best alternative sometimes!!

A2: I think the aim of the question is to discuss the effect of more than 24 hr on the diffusion of the product & standard, so on the size of inhibition zone.Investigate this during validation and if you find no effect take the reading next morning.

ATCC Reference

I bought 5 swab sticks (as 1 package per order) ATCC reference pass.2 but I'm still confused on what to do with all the swabs after I confirmed it as the correct microbes. Should I transfer all the swabs and use it as a culture stock (i.e. store in -20C) ? If that so when will be the good transfer period as the expired date is mostly not more than 6-8 months after arrival due to the shipment ?

I'd really appreciate if anyone can share their experience how to handle the ATCC reference.

Particulate Matter in Ophthalmic Preparation

I want to know if anyone has an experience on which supplier that has a system / software to conduct a particulate matter testing in ophthalmic solution as stated in USP Ch. 789 ? The current software from my current supplier only available to Ch. 788.

A1: The software for the analysis of ophthalmic solutions is available in the latest version of Pharma spec 2.2 version. In this regard you need to have a dedicated CPU to upload the software for the USP <789> chapter.

A2: For particulate matter in ophthalmics ; supplier : Mk teknology1, Pvt. Ltd., New Delhi PAMAS refer to http://www.pamas.in

Movement of Sterilized Items

If the articles (say for eg machine parts) are double wrapped & sterilized, do we need to maintain Class 100 (may be using mobile LAF) during its movement (transit) from Steam Sterilizer to Filling area??? The transit area, including the surrounding area to the filling line is Class 1000.

The second wrap on the sterilized item will be removed prior to placing the sterilized item in class 100 (the first wrap will remain till its usage).

BET for Albumin Products

I am having few albumin based products and as I am testing them with LAL test they are passing the test and when it is retested by Pyrogen testing they are failed (i.e. reproducibility is not there). If some can suggest any method to test those products?

Please also suggest how to test blood products for BET?

A1: Albumin can be tested by the LAL test. An appropriate dilution with LAL Reagent Water usually eliminates the interference. Although the appropriate dilution rate depends on the LAL product, most of (probably all of) commercially available LAL products can be used for endotoxin assay in albumin. Beta-glucan contamination is often observed in albumin preparations. However, it causes only false positives.

Comparison of the LAL test and the rabbit pyrogen test was performed by researchers in 1970's. For example, Mascoli and Weary reported that none of the 81 lots of 25% albumin injection tested was acceptable by LAL but unacceptable by rabbit pyrogen test (J. Parenteral Drug Assoc., 33, 81-95).

In your case, if there is no other pyrogenic substance in the product, immunological reaction may be one of the possibilities. If the rabbits are reused for the pyrogen test of the product, they can have antibodies against albumin which can cause immunological reaction at the second injection of the product containing albumin. Did you obtain the positive results with fresh rabbits?

Preservative testing

How do you prepare initial sample of paper with glue(with preservatives in it) for preservative time kill testing at 30, 40 and 60 min. How do you introduce organism on that glued paper and test to see if they are killed by the preservatives.(usually used in drapes).

Microbiological Testing of API and Raw Materials

I will like to know the practices followed for testing of API and Raw materials. Is the analysis done container / Pack wise or It is done on composite sample?

Training qualification for EM sampling technicians

I am looking to find out and learn about current practices and set-up for training qualification of Environmental Monitoring Sampling Technicians in the aseptic processing industry. If I could understand how this is being done in other companies it would be helpful for me to come up with a plan-

1. What does the initial qualification comprise of and how is this evaluated? Any specific areas and or categories that are evaluated??
2. Is the qualification done as part of routine monitoring? or is this done outside of the routine areas, in a hood etc?
3. Need to know at what frequency do the sampling technicians get re-qualified?
4. Does the re-qual include each and every aspect of sampling (NV, V, S, PM)
5. Is the re-qual done as part of routine monitoring or through special sampling and or watching videos of sampling technicians?
6. Any checklist or bullet points on the different aspects that need to be evaluated.

I would appreciate as much details as possible. Thanks a lot for your help and input in advance.

A1: I suggest a training module should be created with the emphasis for environmental monitoring and related SOPs, methods specs, reading of plates, cGMP guidelines, Rules of conduct in the aseptic area and any videos that detail slow deliberate movements. Do you have videos of "smoke" studies in your aseptic area(s) that you could demonstrate the Laminar flow , and the slow deliberate technique of analysts, production in motion?

This training module should include the gowning qualification of the individual. Some facilities require documentation of training for steps to the initial gowning test(s) by a trainer to evaluate technique and troubleshoot / forsee any problems thereafter.

Key is to document in an organized form (s). Read and document sops, a dedicated trainer for an individual or individuals (one person demonstrating, teaching a uniform method of collection, incubation and recording of results to all trainees).

Another key is to train in the prep areas (non-aseptic) first, gain proficiency and eventually train in the aseptic areas.

The trainer should introduce the new EM person to the sampling sites over a few days, demonstrate the sampling technique, observe the sampling performed by the trainee, and then test the trainee by sampling independently on different days.

The requalification of the EM monitoring was not performed per se from my experience was always performed via annual gowning qualifications, media fill qualifications, ensuring that the analyst's training records via EM were always kept up to date. If a media fill was unsuccessful , potentially those individuals should be restricted form further aseptic activities (Production, EM). The facilities that I have worked always included yearly cGMP training sessions.

A2: 2. Is the qualification done as part of routine monitoring? or is this done outside of the routine areas, in a hood etc?

I didn't answer one of your questions: The qualification of new analysts for EM from my experience was performed as part of routine monitoring with the notation that either the trainee sampled with guidance or independently. The rationale being that regardless of the training...the results would impact the appropriate fill line, production area depending on the sites monitored...Direct critical, Indirect Critical or Other.

temperature mapping/remapping

A couple of questions regarding stability chamber temperature mapping.
These apply to incubators and sterilizers as well.

1) For a qualified piece of equipment, if the temperature is tweaked (controller moved up or down slightly), is a new requalification/temperature mapping required? What is typical in the industry? We feel that the original qualification demonstrates that the unit will maintain temperature +/- Xdegrees around a setpoint and that tweaking within that range does not require additional mapping. Also, the unit is continuously monitored via a separate, calibrated system.

2) If a chamber/incubator/sterilizer door gasket/seal is replaced, does this require additional temperature or humidity (in the case of a stability chamber) mapping?

A1: 1. If you mapped stability chambers at your working range (e.g., 20C, 30C and 45C) you may set any temperature you want between the mapped range. If you did one set point mapping (e.g., 32.5C), changing the set point to couple of degrees is not a problem, and generally re-mapping is not necessary.

2. If you did like-for-like replacement, mapping not required.

Detection limit

I have a vaginal use product which has antimicrobial (bactericidal) activity in 1:10 and 1:100 dilution but hasn't in 1:1000 dilution.
I have applied neutralising agents as Tween 80 and Lecithin. The applied method: pour plate method.
This product is not filtrable
Acceptance criteria: TAMC NMT 100 cfu/g
TYMC NMT 10 cfu/g
Absence of Candida albicans
Absence of Staphylococcus aureus
Absence of Pseudomonas aeruginosa

How should be reported TAMC result on certificate if I do this test with 1:10, 1:100 and 1:1000 and I find nothing?
What will be the detection limit?

A1: Assuming you mean 1:10 and 1:100 do not validate, "acceptance criteria" are inconsistent with your level of detection. The numerical limits as well as the objectionables (absence in x gram) se should be consistent with the 1:1000 dilution (assuming that validates).

A2: When USP or EP recommended Tween 80 (20) and Licithin they followed them with a table of many chemical neutralizers. If you mentioned the name of the antibacterial product or ingredient, it will be easier for us to share you our thoughts. Test other neutralizers with only 1:10 dilution. Then validate it.

Sterility Negative Controls

I know what you are thinking, "not THIS subject again". Actually, I hope that you do not pass up this question without reading further. Rather than discussing the issue with positive results in negative controls, I am asking what might be a much simpler question

In a recent direct inoculation sterility test, the test was performed using the usual two media types (TSB and FTM). However, two different lots of TSB media were used in the same test. The negative control only represented one of those lots.

There is a bit of a debate as to whether both lots required negative controls. Both lots passed department Growth Promotion and Sterility testing. Both lots are visually identical. Does the negative control simply represent an example of "no growth" for the media type, or for only that specific lot?

A1: Each lot needs to be tested! Sterility could be compromised in one lot and not the other for many different reasons!

A2: Oh no not HIM again!

Sorry, I can't resist rising to the bait, especially as we already have a response suggesting that each lot of TSB needs to be applied to the negative control....err, I don't think so!

You've already shown that each lot of medium meets all GPT requirements i.e. they both work. Either one will do. The negative control test is used to show that the environment in which the (Sterility)test, and the operator performing the test might be the source of a contaminated sterility result (see previous thread, and compendia). That's all. It is not a test to show that the media used would permit growth of contaminants...that's GPT, and already done.

What would be the logical extension of performing extra negative controls...just in case you observe growth in a sterility test, and no luck seeing the same bug presenting in the original negative control, well, you might have the contaminant presenting in the second (third? Fourth? Fifth?) negative control with which to "get out of jail"? Sounds like testing to compliance to me!

Incidentally, you're talking about Direct Inoculation sterility testing...so what exactly is the negative control directly inoculated with...?

In a previous life I have convinced Production to perform their own Direct Inoculation Sterility test in situ in the manufacturing Class A filling hood; this had the bonus of not removing the samples from the area, and having to re-enter them into the lab, with possible contamination opportunities, and of course, as fast as possible; get that 14 day clock ticking ASAP. Of course, the use of a Negative Control is completely redundant in that situation; any contamination in any sample or control indicates massive risk that the filling event was compromised.

A3: Te result of a negative control depend of the aseptic condition of the test: environment, sterility of filter, canisters, diluents an media, and the aseptic method related to the work of the operator, specially if the test was not performed into an isolator.

A4: I love the idea of performing direct inoculation sterility test in the filling area. But, what would an auditor think about it? And how would the sampling be done?

A5: First of all according to your standard procedure, why did you include negative control? According to your procedure if you are including to monitor the environment, then it does not matter which lot, but if you are including to test the specific media, I don't see a point because GPT already proved it.

No growth is not for media type, it is no growth for that media type in that environment under those specific conditions.

Again if your procedure states you should include based on a lot and you want to fight based on science, yeah sure you can fight.

A6: As mentioned previously, I have been able to get regulatory acceptance of DI sterility tests. It's not straight forward, as you can imagine; the default method is filtration. (For small devices, DI is a no-brainer, they won't filter; you can also use Flow Path for devices with sterile tubing).

My product was 1 ml human injectable IgGs that were incredibly expensive (~$50/ml) and we were making just 1,000 units per batch, manually filled. We selected the first 5 from the beginning of the run, the last 5, and 10 randoms during, especially any that were suspected of compromise, e.g. if the crimp or stopper was dropped during capping. In this way we were able to convince the auditors that we were looking at the absolute worst case, with a reasonable sample size. A total volume of 20 units was only going to be 20ml, (just enough to wet the two membranes really) and we were reluctant to waste product using a larger volume. In the end the auditor was really quite easy to convince; maybe he was a good microbiologist? In Australia at least, the regulators are quite pragmatic, and acknowledge that they need to support businesses performing this sort of work; if they insisted we Sterility Test 200 mls each batch the product wouldn't be commercially viable.

A7: I think that is simple issue we are discussing again. Actually Negative Control is total control of test or confidence level of test. I do not think anything. Negative is heart of the test.

process validation/ampoules depyrogenation

I would like to ask your opinion about the following:

1. In a validated process when the filter type changes is it required to perform a new process validation? The porosity of the filter remains the same (0,2μ), only the material changes. The process concerns a terminally sterilized solution for infusion. The new filter type has been validated with a sample of our product (only the filtration not all the steps of our production line) from our supplier for extractables and microbial retention.

2. For injectables in ampoules it is a common practice (in our company) to depyrogenate the empty ampoules before filling to ensure low endotoxin value at the end product. For injectables in bags the depyrogenation of the bags is not possible because of their material which cannot withstand high temperature. If the primary packaging material cannot be depyrogenated how can you assure that the end product has low endotoxin value? Both of the above formulations concern terminally sterilized injections.

And a last question: do you think that only sterilization of ampoules used in a terminally sterilized injection before filling is sufficient/acceptable?

A1: A sterile vial is not necessarily free of endotoxin. A properly depyrogenated vial will also be sterile. It will remain sterile if you handle it right. Example, use steel trays/covers that allow transport from the oven to the Class 100 room. Ideally, your oven has two doors and opens into the Class 100 room.

Plastic components are free of endotoxin when molded/extruded. The heat of extrusion is very high. Most endotoxin in plastic materials comes after molding/extrusion. It could be that the bag has been handled well and is still free of endotoxin. Did you try testing empty bags? Put some LRW (37C) in the bag, let it sit with occasional shaking for 1 hour. Test the LRW. Keep the volume of the LRW as small as possible so that trace levels of endotoxin are not "lost" by dilution.

drying effect of media plates

How forum members are addressing the issue of drying of media plates for environmental monitoring?

A1: You can change settling plates each one or two hours but record the result per 4 hours or you can add glycerin to the media 0.1%(v/v) before autoclaving. But should validate this addition.

A2: We perform growth promotion on settle plates after they were exposed to LAF air for 2 hours. Maximum opening time in routine testing is therefore 2 hours.

A3: The fill volume and the incubation conditions do marginally reduce the water activity of commonly used microbiological media. See Garcia de Fernando et al, 1992 Food Microbiology 9:77-82

For example, nutrient agar initially with a Aw of 0.997 has Aw of 0.989, 0.0.989, 0.993 and 0.993 when filled a volume of 10, 15, 20 and 25 mL and incubated for 72 hours at 32 degree C.

These are not changes of practical significance.

In my lab I demonstrated that significant changes in % weight of media in EM plates exposure in a laminar flow hood had no effect on the growth promotion capabilities of the media.

Basically this is a non-issue.

A4: Building on [name redacted] comments - the authors in the following reportedly observed Aw to fall in incubation but not enough to make a difference for high Aw media such as nutrient agar. The authors reported that for media with initial (as made) low values (Aw < 0.94), "... Aw decreased sufficiently to adversely affect the growth of micro-organisms...". they also observed, as Tony mentions, that the greater the fill volume, the less the Aw fell in incubation.

Changes in water activity of selected solid culture media throughout incubation
... de^ Fernando, O Diaz, M Fernandez, JA Ordonez - Food Microbiology, 1992; 9:77-82.
The water activities (a w) of selected culture media commonly used in food microbiology have been determined and the effects of the volume of medium poured in Petri dishes, temperature and time of incubation on a w reduction studied. The a w values consistently fell …

A5: As the EU settling plate requirements are expressed as cfu/4 hour exposure why did you not run the test for 4 hours as I did.

A6: I agree to [name redacted] suggestion of adding 0.1% glycerine to the media and exposing it for 4 hours. However the growth promotion capacity of such media has to be validated. In addition, if your present exposure time is 2 hours, then a comparative study of the 2 hour exposed plates and 4 hour exposed plates (with glycerine) can be an additional data that could justify the change from 2 hours to 4 hours.

A7: I think the supplier recommended maximum opening time of 2 hours. Therefore 2 settle plates opened for 2x2 hours are exposed.

A8: Then change your supplier or increase the fill volume as changing plates after 2 hours is an unnecessary intervention.

A9: Is there any reference for addition of 0.1% glycerin to the media to avoid media dryness????

A10: I agree with [name redacted]. For air monitoring plates adding more agar will prevent drying.

A11: I agree, but our fill processes do mostly not last longer than 1.5 hours.

fire sprinklers cleaning

I would like to ask about your experience with fire sprinklers cleaning.

In my plant we have a new system and after few months, especially at department of solid dosage forms (where is a lot of powders), we have problems with cleaning them.

Do you have any procedures of sprinkler cleaning, cleaning frequencies or maybe you have some solutions to protect sprinklers from powders and dust.

USP Monograph 1117

I am currently studying for a Pharmaceutical Microbiology MSc and have been set an assignment which reviews a USP general chapter <1117> Good Pharmaceutical Microbiology Laboratories best practice. We do not have access to the current USP in our laboratory, and as far as I can tell I would have to purchase the whole USP to gain access to it. Does anyone know of anywhere you can purchase / view individual USP chapters?

A1: Most college libraries have the USP.

Cleaning validation of tablet press

Is it possible to perform swab testing of tablet press. if yes then is there any developed method for swab testing?

Determination of Fiducial limit in Microbial Assay

Can anyone inform me regarding calculation of fiducial limit of Microbial Assay of antibiotics?

A1: You establish that when you validate your plate reader

Development of In-Process Bioburden Limits For Bioprocess-Derived API

Is there any guidance or references one could provide with regards to developing limits/specifications for in-process bioburden monitoring of bioprocess API production? This is for a bioprocess running under non-sterile conditions during protein purification process steps. Our process is typically very low in bioburden (only 3.3% of our submitted samples have any detectable bioburden (per USP 61 filter method), 33 out of 998 samples) and if present it is often only one or two colonies. Given the predominance of "zeros", effectively within the “noise" of the assay, is it even possible to consider setting limits? If one were to set limits, given this historical dataset, what would be the best approach to set limits for each process step (column chromatography, tangential flow filtration, nanofiltration, etc.)?

A1: There are two major considerations, i.e. the bioburden that would have the potential to add threshold levels of microbial-derived toxins and challenge sterile filtration steps.

Pre-sterile filtration bioburdens are driven by EU GMP requirements, i.e. NMT 10 cfu/100 mL, whereas endotoxin threshold are at or above 10^3 cfu/mL.

Biologics companies have received regulatory citation for bioburden control of in-process materials but these are often related to exceeding in-house specifications, inadequate investigations and related lack of sterility assurance.

As the actual in-house levels in 483 citation are abdicated it is difficult to determine the levels used by different companies.

A2: Thanks [name redacted, but is not the 10CFU limit more to do with pre-filtration of drug product and not for in-process analysis during the drug substance (API) production/purification? Are there some good references that speak to how to go about setting the limits (bioburden and endotoxin) for in-process samples of the purification steps (column chromatography) for a bioprocess?

Thus far I've seen, as you've suggested, “use the mandated drug product in-process limit of 10CFU”, or “use three standard deviations” (although this is hard given the prevalence of "none detected" results), to “anything less than TNTC as the process had proven capable of removing the organisms and related toxins". I'd like to think there is some in between approach that is more commonly used for this specific in-process application.

Microscopic inspection for bacteria

Are there any references as to the concentration of bacteria needed to reliable detect (i.e., be certain what you are looking at is a bacterial contamination and not just debris) under microscopic examination of 400-1000X?

A1: Appl Environ Microbiol. 1982 August; 44(2): 376-382

Statistical analysis of the direct count method for enumerating bacteria.
D Kirchman, J Sigda, R Kapuscinski and R Mitchell

A2: Thanks [name redacted]. I reviewed this paper and although it speaks to using a microscope and the relative variance of counting the organism, it does so on filters and does not address my initial question. I wish to know if (there very well may not be) there is a good reference as to the concentration of bacteria (e.g., >10^3, or >10^6, etc...) needed to reliable detect in a wet mount of a mammalian cell culture sample under microscopic examination of 400-1000X?

A3: Sorry for a slow response, but I believe that you will not find any references. The closest you may find would be for counting blood cells. It has been so long that I don't remember the calculations. You could back into a theoretical limit based on the volume you are viewing then set up some studies to count in parallel with plate counts to establish a range.

enrichment of Yeast

After a sample is diluted 1:10, then I incubate it at 35ºC for 24 hours in order to determine absence or presence of Pathogens. Then I pass a loop to a selective or differential media. Well, if I need to find Candida albicans, and I incubated first at 35ºC and the selective media is incubated at 25ºC, maybe the yeast was killed because of high temperature in the first step, so my result could be a false negative. I have follow this methodology for long time, because It's takes to many days if I incubated at 25ºC for 4-5 days for the enrichment step. So my question is how to do the enrichment in order to recuperate Candida and at the same time, I do not need more days for the results?

A1: For information regarding the absence testing of Candida albicans, the USP <62> gives you all the information that you needed.
From experience, we always had a recovery of Candida form the media (SDB) that was incubated in 35 degree for 3-5 days or less than three days.
To find a clear perspective regarding this issue, I think you should perfom a study and you will see the outcome.

A2: Candida albicans don't die at 35ºC. For a growth promotion test for TSA, you have to incubate C.albicans <5 days by 30-35 ºC.

I'm not sure if 24h is enough for the enrichment step. Hopefully someone else can help you with that question.

A3: Thank you for your information, It helps me a lot. So I have to change the method using Sabouraud Dextrose Broth. 24h it's not enough, so I have to standarize the method by 3 days incubation.

Purified Water testing

Do we do color and appearance test for purified water samples? If so, can I get some details about the method? I know that there are compendial methods for color and clarity in EP and USP for liquid samples, but I never heard about purified water color and appearance test?

A1: Color and clarity of purified water, you have to inspect the appearance characteristics of purified water visually for clarity (no turbidity) and color (colorless) I think this is enough.

Monitoring of gloves of the glove ports

Do we have to monitor the gloves of the glove ports at the end of the dry powder injectable filling???

If yes, is it to be monitored as "finger dabs" or take swab from individual finger of the glove & monitor it individually or make a composite of all the fingers & monitor it???

A1: Suggest you monitor the gloves individually. Swab is preferable 1) so as to sample more area 2) to eliminate the residual media traces issue associated with the finger dab technique.

A2: But these gloves are then sterilized. Will taking swab individually & plating them individually increase the total no. of plates???

A3: To monitor the gloves of the personnel involved in the aseptic activities, we may prefer to sample the finger dabs. But in case of isolator gloves, it is preferable to swab these gloves rather than finger dab. You need to monitor individually. This will help in case of any issue with the gloves and aids in investigation.

A4: I do agree with [name redacted] h as if you do finger dab technique there may be chances to have traces of media to the gloves which may leads to contamination and its a risky factor for sterility.

But if your Isolator has been sterilized with VHP and if you are doing environmental monitoring like air sampling, glove monitoring shall be avoided based on the trend analysis

As in the isolator i suggest to have less men interventions of course it shall depends on complete Risk analysis,Impact analysis and Risk mitigation.

INDEX NER OF SPECIFICATION

I need to know your experience about microbiological purity of non-sterile products, particularly when you have index ner of specification. For example TYMC 80 and after repetition it's 70. Is it alarm level? what present of specification considers as alarm level? please share me your experience.

Media Fills

I am new to sterile processes. I want to understand requalifications in media fills. If we have say two type of products with common filling machine (Ophthalmic & Small volume injections). Also size of container used in ophthalmic is from 2 to 10 ml & vial is from 2 to 20 ml. Can i plan media fill in routine revalidation (6 monthly) like-

First six monthly- smallest container at worst speed of machine for both type of product
Second six monthly- Largest containers wiht slowest speed.

This way i am covering extremes in whole year.Is it acceptable to regulatory.

Also it there any formula/procedure/guidance which specifies for what batch size, how many containers shall be filled. Regulatory guidances says <5K or 5-10K or >10K, but do not specify any relation with batch sizes, because it is not possible to take media fill equal to actual product batch size.

media fill

In september 2004 USFDA guideline mentioned the following lines.

When filling from 5,000 to 10,000 units:
One (1) contaminated unit should result in an investigation, including consideration of a repeat media fill.
Two (2) contaminated units are considered cause for revalidation, following investigation/

kindly give your valuable comments in first case we can repeat the individual media fill, but in second case what we should do in revalidation?

A1: Does anyone knows the service , calibration, pm and parts support of VHP 1000 other than the Steris company or any other company manufacturing the compatible equipment to VHP 1000 Biodecontamination system.

A2: In case of two contaminated units detected in the media fill when filling from 5000 to 10000 units, the accepted practice is : if the investigation could find out an obvious/exact root cause and preventive action is taken to eliminate it, one successful media fill is enough. If the root cause could not be established, then the corresponding filling line should be re qualified with three media fills. the same approach is applicable when filling more than 10000 units.

A3: Besides Steris there's Bioquell:

http://www.bioquell.com/

A4: You might try Agape Instruments (www.agapeinstruments.com). They are located in Cincinnati. I have used them for years in hospital, pharmaceutical and industrial microbiology settings. Their prices are reasonable and their service is excellent.

A5: Health Canada

The table indicates the maximum permitted number of contaminated units per
various Media Fill "run sizes" to indicate a 0,10% contamination limit with
a 95% confidence level

Contaminated units permitted ((action level)	Filled units per run
0	3000
1	4750
2	6300
3	7750

4
9150

5
10510

6
11840

7
13150

8
14430

9
15710

10
16960

Question for Disinfectant and its Efficacy specialist

I have following questions regrading efficacy methods of Veterinary Disinfectants which can be used in veterinary hospital as well as animal housing. The proposed efficacy test methods are based on the situation – Vet hospital vs Animal housing. Vet hospital are being considered 'clean environment' as compared to 'animal housing'. The following standards methods have been proposed with some modifications:

For veterinary hospitals ----Clean conditions:

1. ASTM E 2197-02, Standard Quantitative Disk Carrier Test Method for Determining the Bactericidal, Virucidal, Fungicidal, Mycobactericidal and Sporicidal Activities of Liquid Chemical Germicides - Staphylococcus aureus(ATCC 6538), Pseudomonas aeruginosa (ATCC 15442), Salmonella typhimurium (ATCC 10708), Escherichia coli (NCTC 8196), Canine Parvovirus Cornell strain (ATCC VR-2017), Trichophyton mentagrophytes (ATCC 9533), Bacillus subtilis (ATCC 19659)

2. The efficacy testing requirements described in Australian Therapeutic Good Adminstration 'TGA Disinfectant Test' (Kelsey- Skyes test) for clean conditions only- Staphylococcus aureus (ATCC 6538), Pseudomonas aeruginosa (ATCC 15442), Salmonella typhimurium (ATCC 10708), Escherichia coli (NCTC 8196). http://www.tga.gov.au/docs/html/tgo/tgo54.htm

3. EN 1040, Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of basic bactericidal activity of chemical disinfectants and antiseptics – Test method and requirements (phase 1) Staphylococcus aureus (ATCC 6538), Pseudomonas aeruginosa (ATCC 15442), Salmonella typhimurium (ATCC 10708), Escherichia coli (NCTC 8196)

Animal Housing- Use dirty conditions

1. ASTM E 2197-02, Standard Quantitative Disk Carrier Test Method for Determining the Bactericidal, Virucidal, Fungicidal, Mycobactericidal and Sporicidal Activities of Liquid Chemical Germicides - Staphylococcus aureus(ATCC 6538), Pseudomonas aeruginosa (ATCC 15442), Salmonella typhimurium (ATCC 10708), Escherichia coli (NCTC 8196), Canine Parvovirus Cornell strain (ATCC VR-2017), Rotavirus Wa strain (ATCC VR-2018), Vaccinia WR strain (ATCC VR-119), Trichophyton mentagrophytes (ATCC 9533), Aspergillus niger (ATCC 16404), Bacillus subtilis (ATCC 19659)

2.The efficacy testing requirements described in 'TGA Disinfectant Test' for dirty conditions Staphylococcus aureus (ATCC 6538), Pseudomonas aeruginosa (ATCC 15442), Salmonella typhimurium (ATCC 10708), Escherichia coli (NCTC 8196) http://www.tga.gov.au/docs/html/tgo/tgo54.htm

3. EN 1656, Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in veterinary field – Test method and requirements (phase 2, step 1) Staphylococcus aureus (ATCC 6538), Pseudomonas aeruginosa (ATCC 15442), Salmonella typhimurium (ATCC 10708), Escherichia coli (NCTC 8196)

Virucidal Tests for Vet Hospitals: clean condition
ASTM E 1053-97 – Standard Test Method of Virucidal Agents Intended for Inanimate Environmental Surfaces

Animal housing : Use dirty conditions Canine Parvovirus Cornell strain (ATCC VR-2017), Rotavirus Wa strain (ATCC VR-2018), Vaccina WR strain (ATCC VR-119)

Fungicidal for veterinary hospitals
1. AOAC Official Method 955.17 Fungicidal Activity of Disinfectants using Trichophyton mentagrophytes
OR
EN 1275, Chemical disinfectants and antiseptics — Quantitative suspension test for the evaluation of basic fungicidal or basic yeasticidal activity of chemical disinfectants and antiseptics — Test method and requirements (phase 1) Trichophyton mentagrophytes (ATCC 9533

Fungicidal for animal housing
Trichophyton mentagrophytes (ATCC 9533), Aspergillus niger (ATCC 164042. EN 1657, Chemical disinfectants and antiseptics–Quantitative Suspension Test for the evaluation of fungicidal or yeasticidal activity chemical disinfectants and antiseptics used in the veterinary area – Test method and requirements (phase 2, step 1)

Sporicidal for veterinary hospitals
AOAC Official Method 966.04 Sporicidal Activity of Disinfectants Method II
OR
EN 14347, Chemical disinfectants and antiseptics — Basic sporicidal activity — Test method and requirements (phase 1) Spores of Bacillus subtilis (ATCC 19659 or ATCC 6633)

Sporicidal for animal housing

EN 13704, Chemical disinfectants – Quantitative Suspension Test for the evaluation of sporicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas - Test method and requirements (phase 2, step 1)

My questions are:
1. How comparable these tests are with each other? Will they give same end results? : I think they are not comparable.
2. Is there any need to validate these tests since the proposal is to modified the test methods in terms of the number of log reduction is now 10 ^ 6 (standard test described 10^5). The proposal is to change the organisms too. - I think any modification in standard method requires validation.
3. If somebody can tell me the difference between phase 1 and phase 2 test of EN test series.

Laboratory Dishwasher

We are looking into getting a new laboratory dishwasher for our QC Micro lab. Does anyone have any experience with the Lancer Model #815 LX or 820 LX?

A1: We have an older Lancer Model# 810 UP, and the manual describes it as "one of the most perfected machines in existence". This doesn't really help with your question, but it is amusing. We've never had a problem with our dishwasher though, if that does help at all.

reportable result

Could somebody help me.
The API is Terbinafine HCl. This API is a fungicide.
Tween, Lecithin , pH adjustment, dilution, membrane filtration dosn't work.
The results of microbial enumeration test (TYMC):
Pour plate : 1:10 , 1:100 and 1:1000 : negative

How should I report the TYMC results for a routine test?
What is determination limit in this case?

Fungicidal product

I have a vaginal product with fungicidal property.
I couldn't recover Candida albicans from 1 g according to test for specified microorganisms.
Tween, Lecithin and membrane filtration does not work.

How should I report the result of test for Candida albicans on Certificate of Analysis?

My idea:

Test	Requirement	Result
Candida albicans	Absence in 1 g	not detected* in 1 g (*: because the product is fungicide)

Is it acceptable?

A1`: The harmonised methods state that 'if for a given product the antimicrobial activity with respect to a micro-organism for which testing is prescribed cannot be neutralised , then it is assumed that the inhibited organism will not be present in the product.' A result of not detected in 1 g should be sufficient.

A2: The question then becomes, How far do you have to take the neutralization? We currently have a product we test using a customers "special" buffer we can purchase by agreement only. Our test has never been able to recover Bacillus even with neutralizing broths and dilution at 1:100, but their "special broth" allows us to use 1:10! No, they will not tell us what they have discovered, and they are the customer…..

Lucky for us it is a food grade product not a pharmaceutical product and we can have a "bacillus" plate count of up to 20,000cfu/g for this product.

Detection limit

I have a product with antimicrobial activity in 1:10 dilution.
Applied method: pour plate method.
Applied dilutions: 1:10 and 1:100

How should I report the result if I didn't find any colonies in 1:10 , 1:100 dilutions?

Which is the correct solution? : <10 cfu/g
or <100 cfu/g

A1: the correct is <10 cfu/g.

A2: No matter amount of Diluent used, since diluent appears sterile it should be reported to the pharmacopeial limit whether 10 cfu per gm or 100 cfu per gm as in you spec. if you feel antimicrobial activity is not neutralize fully it can be diluted further.

A3: If the product had passed the method suitability test with a 1:100 dilution, you have to report the results of the 1:100 dilution.
The LOD for pour plate is <100 cfu/g, so you have to report <100 cfu/g if you find no colonies.

The results of the 1:10 dilution are invalid because you see antimicrobial activity. So your method suitability test failed by this dilution, and this dilution is unusable for a good interpretation for your TAMC and/or TYMC count of the product.

If you only see inhibition by TAMC and not by TYMC by a 1:10 dilution, you can do the method suitability test for TAMC with a 1:100 dilution and TYMC with a 1:10 dilution.

A4: If it is confirmed by you during validation that your product shows antimicrobial activity in 1:10 and this activity is neutralized in 1:100 dilution.
You should perform testing at 1:100 dilution and report <100cfu/g if you do not observe any colony.

A5: You need to write <100 cfu/g, because if the product has antimicrobial property , it has to be analyzed at higher dilutions, and in case if you want to report <10 cfu/g , increase the plates to 5 sets and analyze 2 ml /plate and summerise and report.then apply 1:10 dilution factor for calculation.

A6: I totally agree with [name redacted]. Since from the method suitability you found out that 1:100 dilution is valid you should use this dilution for MLT of this product. If you find no colonies then you should present your result as <100CFU/g.

A7: How can you say <10 cfu/g if you have an antimicrobial activity in a 1:10 dilution?! That's a very wrong interpretation!

Marianna, please look at the validation documentation of your product. Your method suitability can tell you everything about the growth- and inhibition properties of micro-organisms in your product and dilution.

It's very important to do a method suitability test. With no method suitability results, you can say nothing about your results!

Particle counter calibration

We are using the Met one model (3313) of particle counter to monitor our Glass C cleanrooms. We recently has a calibration supplier visit who expressed a concern about this model of counter passing the current draft revision of ISO 14644, which will require the user to use the calibration standard titled 'ISO-21501-4 Determination of particle size distribution-single particle light interaction methods- Part 4: Light scattering airborne particle counter for clean spaces'. Apparently, the majority of air particle counters manufactured before 2007 will not have been designed to pass the more stringent calibration and verification laid down in ISO 21501-4. The timeline for publication of the revised ISO 14644 is early 2011. My question (s) to the forum : How are people using pre-2007 manufactured particle counters plan to handle this change to the standard?

A1: I dont think there is anything in 21501-4 that would make 3313 obsolete. As far as calibration of the counter goes, the procedure as specified in 21501-4 does not say anything that was not being done by Met One or some other manufacturers. 21501-4 asks you to size calibration.

What is new is counting efficiency check. This requires comparison against a more accurate device for the smallest channel and the next size. Again this is just a check and an external one.

Your supplier's statement can be said to be true if the unit were to be Climet or PMS as their calibration procedure was different.

Nothing like buying your own copy of 21501-4

A2: We have been informed by Hach Lange a supplier of Met One particle counters that the 3300 series will not meet the ISO 21501 standard. We also have 3400 series onsite which will meet the standard and we will use going forward in classified areas. Has anyone else come across this with regards the 3300 series Met One particle counters not being suitable for meeting ISO 21501 standard?

Isolation of Stress microflora

Can we expose low nutrient agar media (NAM / PCA) in our sterile critical area (100A or 100B) of injection to isolate the injured microflora that couldn't be grow on SCDA or SCA media?
Will it beneficial for isolation of such microorganism?
Does any pharmacopoea or guideline refer it?

A1: You could certainly do so, but I would ask why you would want to? The point of environmental monitoring is to monitor the "state of control" of your facility - all monitoring has meaning only in relation to other monitoring results. It is always possible to set out different media to determine the presence of organisms capable of growing on that media, but how will you use this data? Will it be used to monitor the facility or the product? I would strongly urge you to consider the rationale before assuming that EM data tells you anything about the finished product.

A couple of interesting articles on this issue:

Hussong, D and RE Madsen. 2004. Analysis of Environmental Microbiology Data From Cleanroom Samples. Pharm Technol. Aseptic Proc:10-15.

Farrington, JK. 2005. Environmental Monitoring in Pharmaceutical Manufacturing - A Product Risk Issue. Amer Pharm Rev. 8(4):26-30

I guess my response would be that you can certainly do this, it is not required, and I personally question the purpose of this monitoring. I urge you to carefully consider the rationale for performing this monitoring and how collecting the data will benefit your operation - how will the data be interpreted and used?

A2: I recently asked a question about isolating anaerobes from inoculation as well as incubation room of a microbiology laboratory. is it required to isolate anaerobes from environment and what will be the media recommendations for that.

Bioassay equipment

We use agar diffusion assays to test the potency of antibiotic in our formulations.
Agar is poured into 12"x12" glass plates.
The equipment supplier, Scientific and Technical Supplies appears to be no longer in operation (www.stscambridge.co.uk) therefore if the zone measuring device broke down, we would be unable to track spare parts. The zone reader magnifies the zone image approx 7.5 time and sends this info via a RS cable to the computer program.

Does anyone have an alternative source for similar equipment?

A1: We have "latin square" method for bio-assay - technology (reader) is from Synbiosis - so also agar diffusion method (6*6 wells in the plate - 6 times the unknown dilutions and 6* the standard dilutions). We work at 3 concentrations/dilutions : 12µg/ml, 6 µg/ml and 3 µg/ml.

Let me also pose a question to the forum.

We have validity rates of the test of 50-70%. Typically we fail the linearity criterium.

Can other members with similar bio-assay method share there validity rates (how many plates are valid at readout / total number of read plates) ?

We have done already a lot of study work and did not succeed in increasing validity rates yet so it would be interesting to share some thoughts on this subject.

A2: You might want to consider Giles Scientific product (ww.biomic.com) or Synbiosis, Protocol 2 (www.synbiosis.com).
Both of them are computerized system. Hope they can help you.

A3: I am doing microbiological assays (Antibiotics, Vitamin assays) and zone inhibition and exhibition readings are measuring with *Vernier Caliper* if Zone reader is not available.

A4: Are you doing assay for vitamin B 12 . what color of zones do you obtain for this assay if ATCC 11105 is used for the assay. and if you get same sample for consecutive 3-5 days did you obtain same results. I am not getting same results for the same sample. facing lot of problems.

Can you tell me what are the most probable reasons for the erratic behavior of this assay… and also is there any substitute to this culture.

A5: I am doing B12 assays regularly,I get pale clear distinguishable exhibition zones.may be you are over heating the assay agar which leads to charring of media.use clean dried pipette for diluting sample and standard (Preferably use pipetman and Make sure the tip you are using is not sticky inside and releasing solution completely).

How you determine the concentration of standard B12 and this could be the solution for not getting the consistent result, even i faced this problem.

A6: On a related topic, are there any guidelines for biological assays, mainly antibiotic assays for stability testing concerning the assay value being no more than 5 % difference from the previous time-point? This is an ICH guideline for chemistry assay values for stability testing. I am wondering if there is a similar guideline for antibiotic assays, hopefully with a wider spec that reflects the greater variability of biological assays.

A7: Yes i am doing B12 assay for Multivitamin tablets and syrups, i am getting light white colored zone Exhibition, and uncertainty in ur result may due to the sample preparation or age of the culture. To avoid this use same aged culture (4/8/12 hours enriched culture).

A8: I do Cylinder plate assay as well as cup plate assays for antibiotics and B12, I measure the zone size by Zone reader which is really time consuming. Can you suggest me any technology to read zones in 100 mm plate. Preferably dealer should be from India.

A9: Significant change criteria (5% assay change from previous time point) is not applicable for biological assays. Vitamin B12 assay is notorious for its variability, antibiotic assays are slightly better. Reading zones of inhibition or growth using calipers is very subjective.

If overages are added to the product (e.g, vitamins), check possibility of using "5% decrease in assay value at 3 continuous stability intervals" as a significant change criteria.

Monitoring Sterility Test Incubators

We used to monitor Temperature and Humidity (if required) for sterility testing incubators routinely. how about determining the microbiological air quality inside the incubators, since the incubators are opened and closed daily for monitoring the sterility test tube. is their any specific requirement to monitoring requirement for incubators?

A1: Typically sterility testing media is closed container so all other parameters than temperature is irrelevant.

A2: There is no specific requirement to monitor microbiological air quality inside the incubators because all sterility test tubes are completely closed or air tight. So any microbial contamination chances are not there. But we can keep incubators at least in clean area instead of classified area.
Stainless steel caps or autoclavable plastic caps (12 mm/ 18 mm/ 25mm /38 mm etc.) are commercially available for sterility test tubes.

A3: Can you give me vendor details of that autoclavable plastic caps, we do both open and closed sterility testing.Growth in tubes is always been doubtful as the incubators are in 10000 area because of door opening and closing issue.But no problem with canisters. Wrapping tubes with foil or parafilm is acceptable or not and regular sterility Direct inoculation Method users may clarify my doubt.reference for this is most welcome.

ATCC Culture

I'll appreciate if you can tell me how the number of passages are done for the ATCC cultures during its maintenance.

The lyophilized ATCC culture received is itself considered as 1st passage or the reviving done after receiving is considered as 1st passage.

A1: I have attached a link to the ATCC technical bulletin on passages.
It addresses your questions.

http://www.atcc.org/Portals/1/Pdf/tb06.pdf

A2: Depends on your application, but refer to current USP <1117> Microbiological Best Laboratory Practices for general guidance. I don't have it in front of me, but USP <51> on AET testing also has requirements set forth. I believe is NMT 5 passages. You are correct in that the initial thaw is often considered passage #1, that is if you had previously grown the "pellet" up and then aliquoted into seed vials in a cryofreezer (preferably at -70C). You then have 4 consecutive subculture events from the initial thaw. I do not think the USP in chapters 61/62 make mention, but the general practice from the AET chapter seems to predominate across all applications in the cGMP Micro lab.

See this Technical Bulletin from ATCC. They concur with the longstanding USP requirement of NMT 5 passages.

http://www-fhs.mcmaster.ca/safetyoffice/documents/ATCCHighPassageBulletin6.pdf

A3: The ATCC cultures in lyophils shall be considered as Passage 'O' as recommended by the ATCC Technical Bulletin. Revival of the lyophils is not considered as passage. Any transfer of viable organisms on to a fresh media shall be considered as passage. Hope this clarifies your question.

A4: The first culture will be the same passage as state on its label. After you transfer your culture in different media, than it will be 2nd passage.

I personally preserve my culture in beads (Microbank (R)) because my lab. don't have facility to support preservation under N2 supply. You'll need a cryopreservative chamber if you want to use this method and I think this method is the best as it will safe your cultures up to 30 years.

What I'm doing with my ATCC culture after it arrives is to grow on agar, identified and put in beads. Culture from agar also transfer into slant for 2 weeks as a working culture. Every 2 weeks, I subcultured the 1st slant into another slant as a working culture for the last 2 weeks during the month. And every month I transfer another 1 bead and repeat the process again. This is works for me for about 4-6 months, but currently, the beads won't grow sometimes.

A5: Revival and transfer of the lyophilised culture to a fresh slant is considered the first passage. Transfer from this first passage to another fresh medium say after 6 months is the second passage and so on.

A6: of cells from an existing culture to fresh growth medium. Each transfer (subculture) is counted as an additional “Passage".

ATCC defines the first passage as the first broth or agar culture started from the culture vial supplied by ATCC.

This culture could be the Master culture prepared in-house in your lab (in form of freeze dried ampules) or could be the reference culture vial obtained directly from ATCC or other recognized culture collections.

It is recommended not to exceed 5 passages from the original ATCC vial.

A7: Thank you very much for the reference on 5-passage rule for maintenance of reference strains. I have a few questions about how ATCC maintains their reference strains.

I am just curious how does ATCC ensure that their seed lots will not run below Passage 0? And has any reference strains seed lots ever had to be replenished due to demand? If so, how does ATCC deal with additional passages prior to the end-user receipt of an ATCC culture?

Also, in preparation of ATCC's initial seed lots, how many passages are typically used to prepare lyophilized cultures from isolates received?

A8: Good question [name redacted]. As the USP bugs themselves are each ~60 years (or more) form original submission to ATCC, one has to assume they're working from some more recent seed lot multiple passages from the original.

A9: When you receive a culture from manufacturer, it usually certifies the passage. And from here you move ahead, and use it till 5 passages.

A10: Kindly go through the following link and I think this would help you,

http://www.atcc.org/Portals/1/Pdf/tb06.pdf

SAL TEST DETAILS

What is sterility assurance level? how to calculate the microbial load after sterilisation.....any method? I want details about that one.

A1: I would recommend reviewing a basic text on sterilization science - then you might also review the excellent article on this topic by Gregg Mosley at http://www.microbiologyforum.org/news.htm. It appeared in the PMF Newsletter vol 14(5).

A2: Or you can go to PDA.org and purchase either of the books that I wrote on basic sterilization science, one on EO and one on Radiation.

Endotoxin limit

I have got a little lack of understanding about endotoxin limit calculation for parenteral preparation.

I very hope that somebody can help me.

My questions:

1. If preparation could be used by different administration (for example - oral, transdermal, i/v etc.), should we take just the parenteral bolus dose mentioned in the preparation instruction (i/v) for endotoxin limit calculation?

2. If this preparation theoretically could be used i/v and intrathecally, but there is not mentioned intrathecal use in the instruction of our preparation - only intravenous, which value of K (intravenous or intrathecal) should be used for calculation?

3. If product is planned for registration in EC, but using bolus doses and both possible K value - 0,2 or 5 (our product is intended only for intravenous administration, but many other manufacturer indicate intrathecal administration too) I was not able to reach the endotoxin limit mentioned for this product in Appendix E to the FDA Guideline, which limit should I use - the calculated by mine or taken from Appendix or which is less from them?

A1: It seems to me that you do not have an endotoxin question. The endotoxin regs are clear, and you have referred to them correctly. You have either a business decision or an ethics issue. Business decision: are you going to allow intrathecal use? If not, then clearly label the product. Ethics decision: if you will allow the product to be use intrathecally, then use the intrathecal limit.

Abnormal Toxicity

If Abnormal toxicity has to be performed on any Injection (Human dose: 150mg), how calculations are made to determine what shoud be the appropriate dose to inject in mice using 0.5ml of sterile normal saline.

Please let us know the reference of same. (Experts in Animal testing are requested to share their experience)

Sterility Testing in BSC

For those of you doing sterility testing in BioSafety Cabinets, are you doing continuous non-viable particulate monitoring in the hood? Continuous settle plates? Active viable air sampling? How far do you take the stance that the environment/controls must be comparable to the manufacturing environment? Are people monitoring to the EU/ISO/USP standards (as applicable) or are your testing areas actually qualified and therefore classified.

We do our testing in a biosafety cabinets in a "cleanroom". We treat it like it is classified with gowning rules and cleaning/sanitization practices and do viable and non-viable monitoring and trend the data. It is not truly classified though and was not formally qualified to ISO standards. We are getting some push to classify the hoods to EU Grade A and background to EU Grade C. Gowning room I guess would be step down from C to D.

A1: The ideal practice is to qualify the area as per design air classification with consideration to air changes and pressure differential. Before using the testing environment i.e. BSC hood and background area, for routine testing it shall be qualified.

It should be noted that your testing area shall be equivalent or better than manufactuing area.
During sterility testing viable monitoring (Active air sampling, settling plate and surface monitoring) shall be carried out, which enables to identify the root cause of sterility failure. Continuous Non viable monitoring is not mandatory but Hood shall be checked daily/ each shift for non viable particles per meter cube.
BSC hood should comply the ISO 5 requirements.

Validation of Surface Monitoring Procedure

I am working to establish an environmental monitoring program for a start up parenteral manufacturer. My question regards the method validation for our surface monitoring procedure. We will be using both contact plates (TSA with Lecithin and Polysorbate 80) and swabs on the floor, wall, and equipment materials. I have obtained coupons of each type of surface material. My question is what is the best method for validating the procedure. My initial thought was to clean the surfaces per our production SOP, spike the surface with <100 organisms of the "big" five (Staph, Pseudo, Bacillus, Candida, and Aspergillus), allow the spike to dry, and then sample the surface with a specification of at least 50% recovery. However, I feel I will get less recovery because some organisms will die off while the spike is drying. Other thoughts I have had are to sample immediately after spiking without allowing to dry or to "sample" the surface to place any cleaning residue onto the media and then Growth Promote. These options both seem to have drawbacks as to their validity. Finally, I do not see any guidance in the USP as to performing such a study. Is this even necessary?

A1: I don't recommend inoculating in the manufacturing area. I've taken a validation approach to validating EM by doing real time sampling during manufacturing either 3 days in a row or one day a week for 3 weeks. I would do the sampling at maximum production at the same location each time. I'd select a larger number of sites than recommended. That way you get an accurate idea of what organisms are present and how many. I would qualify the agar medium beforehand. You can follow up the initial 3 samplings, with 1 sample a week for a month, then go to monthly.

A2: I wish you the best of luck. I've participated in and done a number of such studies over the decades, and read a number of others- and I believe 50% recovery to be about as attainable as Peace in the Middle East. RODAC plates can produce decent recoveries, provided the inoculated organisms have not, as you suggest, died from desiccation, but with swabs, you will be lucky indeed to see 5%, much less 50%, recoveries. This is good data to have, but the bottom line is, you have swabs and RODAC plates or roughly equivalent methods, and that's State of the Art- this is what everyone uses and is Industry Standard. You may have lousy recovery, but there isn't any way to do better for routine surface monitoring. There are some studies in the literature- I'm sure someone here can provide references, I don't have them at present- but good luck.

A3: Use spores to spike the coupon. If recovery is less than 50% use a recovery factor.

A4: Depending on your method 50% recovery is going to be a challenge. There was a recent report about a new swab that is supposed to boost recovery (I have no personal experience with this product so can't personally vouch for it):

Dalmaso, G., Bini, M., et al. 2008. Qualification of High-Recovery, Flocked Swabs as Compared to Traditional Rayon Swabs for Microbiological Environmental Monitoring of Surfaces. PDA J Pharm Sci Tech. 62(3):191 - 199

A couple of good background articles have recently appeared in response to DOD funding -

Edmonds, J., Collett, P., et al. 2009. Surface Sampling of Spores in Dry-Deposition Aerosols. Appl Envir Microbiol. 75(1):39 - 44

Pinto, F., Hiom, S., et al. 2009. Evaluation of the Effectiveness of Commercially Available Contact Plates for Monitoring Microbial Environments

Letters in Appl Microbiol. 48:379 - 382

The Applied Environ Micro article should be available online.

A few points to be cautious about in designing your study:
1 - Don't set your acceptance criteria before determining the capability of the assay. 50% is, in my opinion, pretty ambitious.
2 - Don't contaminate the manufacturing area. Use coupons of the materials of construction or do the work in unclassified, less critical areas
3 - Resist the urge to put a "conversion" factor on your data based on this study. The important aspect of EM data is the trend, not the absolute numbers. Even in aseptic processing, where we have some scientifically unjustifiable numbers for the aseptic core, these arbitrary numbers were set with the assumption of the recovery capabilities of the methods. For example, let's say your recovery efficiency as determined by this study is 15% (not an unreasonable number for many techniques). If your alert limit is 5 CFU and your action is 10 cfu (hypothetical example), recovery of one CFU would exceed alert (100/15 = 6.7; 1*6.7 = 6.7; 2*6.7=13.4). The "conversion factor" is useful in medical devices sterilized using a "bioburden-based" cycle. Don't misapply it to EM.

A5: There is also another new swab on the market that deserves equal consideration. Data on this swab is presented in the following recent PDA publication:

Rubio, S., McIver, D., Behm N., Fisher, Madeline and Fleming, William, PhD, 2010, PDA J Pharm Sci Tech., 64 (2), 167-181.

There is also information regarding qualification of EM methods in Laboratory Validation, A Practitioner's Guide, 2003, PDA, edited by Jeanne Moldenhauer.

A6: Everybody advised that the recovery should be at least 50%. My question where have mentioned it should be 50% ? Please give us the reference.

A9: You will find that the EM methods you are using do not lend themselves to what would be logical validation methods because the methods are not that good, The revisions to USP <1116> will cover this. Also CDC did extensive method capability studies on surface recovery methods and basically showed that their capabilities do not come close to matching the "specifications" commonly used. There are numerous publications going into method capabilities for EM methods including some I have written. By trying to do surface spike and recovery studies you will only show that your methods are not capable of doing what you think.

USP Water Sanitization

I am interested in what everyone does pertaining to sanitization; if/when their USP water system goes down. I am wondering what the standard amount of time the system may be down before sanitization is necessary. What is industry standard for pharmaceuticals and how did everyone determine timeframes for their systems?

A1: I assume by "USP Water", you mean USP Purified Water. I can't say that there is any standard time or standard practice because there are no standard systems. It all depends on many, many factors related to how the distribution and purification portions of the system are configured and used, whether automatic valves at use points fail open or closed (and how rapidly), whether there were manual valves open and in use at the time the system went down, whether the new pressure differentials (for a non-circulating system that now has NO net positive pressure other than perhaps a hydrostatic head pressure) at use points could allow ingress into the system, etc. Biofilm grows relatively slowly in most water systems (the possible exception is PVC systems), so the risk is not biofilm growth during the downtime as much as it is ingress of contaminants (chemical or microbial).

If the system is completely closed with no use point or tank ingress opportunities at the time of or since the system went down, and it had very low microbial counts when it had been running (<<1cfu/mL) and good ongoing controls like in-line UVs and/or an ozonated tank or continuously heated system, and the company has a high threshold for risk, then 2-3 days or even a week of downtime probably has minimal consequences. If you are not certain about the completeness of the closed condition or have “non-low" microbial counts or have no ongoing microbial controls or have a potential material of construction leachable issue (e.g. from PVC), or your company has a low tolerance of risk, then system flushing and sanitization could be appropriate even after a short downtime of an hour or two.

So it all depends on the water system and management risk aversion as to what should be done. Some companies have actually conducted downtime studies to assess the impact on microbial counts and chemical purity and have developed allowable time windows that way, but most do not. Those that did do these studies probably did them when the system was young and being validated and those data may no longer be applicable to your "mature" water system.

There are just too many factors to give a standard answer, so don't rely on industry precedent -- that's a poor way to run a water system (and just about everything else, for that matter). Your best answer will come from understanding how your water system operates and the associated risks as well as the "risk aversion climate" at your company.

Conservation of A. brasilensis

Usually I haven't had problems with Aspergillus conservation , but recently I activated a new ATCC strain and the mold didn't growth up with the typical morphology. The answer of the supplier was that there was a problem with the petri dishes, because they didn't have vents. Then I activated a new “Kwik Stick" in a petri dish with vents. The mold growth up ver well. But I have a problem because I always preserve the strain in TSA with glicerine. This A. brasilensis strain can't growth up in tubes because of the poor oxygen, and If I left the culture in the petri dish, I can’t preserve the strain for more than one week. I would have to activate a new "Kwik Stick" per week, and this is expensive for my lab. My question is how can I preserve the mold?

A1: I have always found Aspergillus brasiliensis to grow better on Potato Dextrose Agar. Instead of using TSA with glycerin to preserve this organism, I would suggest that you use Potato Dextrose Agar slants in screw-capped test tubes and store several of the sporulated slants under refrigeration (2-8 degrees centigrade) until use. Unlike bacteria, Aspergillus brasiliensis spores on the Potato Dextrose Agar surface should be sufficient for your needs without using a glycerin overlay to preserve them. To prevent desiccation of the slant or water condensation from entering the test tubes, you would just tighten the screw-cap.

A2: My question is why did you choose to use TSA to grow mold!. The ideal growth medium for filamentous fungi are PDA [potato dextrose agar] or MEA [Malt Extract Agar]. TSA is not an ideal growth medium for fungi. Try using other media , that should grow well in tubes.

A3: Hi Vivian,

You could try this :
1. Buy an ATCC freeze dried pellet of Aspegillus brasiliensis (approx $ 155 per vial)

2. Reconstitute this ATCC vial per vendor's directions (vendor directions are provided with the freeze dried pellet , unique for each organism, use recommended volumes , broth culture or rehydration media etc).

3. Using TSB with 15 % glycerol (Remel) , bring the suspension volume to >/= 22 ml approximately (22 ml based on our in-house study gives us the results we need)

4. Make approx. 40 aliquots of 0.5 ml in 1 ml cryovials and store these aliquots at </= - 65 C.

5. Pull representative vials at appropriate intervals and check for vibility and purity of this organism.

This way you can use one freeze dried pellet (approx. 155 $) for almost a year.

If you run out or the viability goes down you would start all over again .

If the purity gives erroneous results , you would have to obviously discard everything and start from scratch.

A4: Asp.brasilensis can be preserved on sabouraud agar slants and transfered every month till you reach the fifth transfer then you can open and activate a new one after that with no problems at all during all transfers.

A5: To conservate strains we use cryobank (copan). these system permits microorganism conservation after activation. this is cost-effective and easy to handle. We use it for all strains in our lab and with this system we have preserved all our microorganisms until its expiration date including A.brasiliensis. After you activate a new Kwik Stick" your microorganism in agar (around 5 days for A.brasiliensis) pick up colonies and inoculate cryobank tubes until the liquid in the tubes become a turbid suspension then mix them to permit the microorganism attaching to the beans inside the cryobank and the discard the liquid and freeze it.

Each cryobank tube has 25 beans and when you need to spike Aspergillus you only with a needle remove a bean and pass it over a Sabouraud agar and incubate for 5 days.

A6: The cryobank definitely looks interesting and and easy to handle.
Couple of questions that I had :
1.What is the storage temperature for these beans?
2. If the beans are frozen down, since each vial has 25 beans and if you used only one bean at a time, could you refreeze the remaining 24 beans? Does that require any additional preparation etc?
3. How about the viability of the remaining beans, does the freeze-thawing affect the viability?
4. Do you also perform a viability and purity check of the organism at frequent intervals?
3. Is there a correlation between 1 bean and the cfu obtained? (as in 1 bean gives you approx. how many cfu?)
4. Is this test only qualitative or can also be used for Quantitative tests as well(that require a certain # of cfu to be inoculated)?
Your response and guidance would be highly appreciated.

A7: I want to share with you some personal experiences which I got when I was working in Mycology and Mycotoxicology Dept. in Veterinary Research Institute a lot of years ago.

Aspergillus spp., Penicillium spp., Mucor spp.and Fusarium spp. are the most spread molds in the environment. Our experience showed that the optimum conditions for these molds are : Czapek agar (with some modifications-adding gentamycine, total agar content have to be 20g/l), pH 6.5-7.5. We were incubating Aspergillus and Mucor spesies for 2-3 days at 35-37 oC and Penicillium and Fusarium spesies for 5-6 days at 25-28oC. We used these conditions to support fast growth and typical morphology of these molds.

Another study : We used Csapek agar to diagnose aspergillosis in poultry. We were putting the pieces of chicken lungs (from died 1-2 days chickens) on to agar and after 24 hours incubation at 37 oC we were observing very good growth and morphology of Aspergillus fumigatus, Asp. flavus and Asp. niger.

Have to note that Czapek is not suitable for yeasts. SDA is good for Candida species and other clinical important yeasts, PDA and MEA are good for yeasts isolated from food. Pathogen yeasts as Candida albicans grow better at 35-37 oC, because this temperature is close to human body temperature (this temperature is valid for incubation of most warm blooded animals pathogens).

Conservation of mold strains : We were using slant Czapek agar in screwed tubes. For long time keeping of molds, an abundant spore formation is more important than water content of agar.
In one study, in slant Czapek agar tubes we inoculated different Aspergillus species, incubated for 5 days at 37 oC and than we kept the strains 2 years at room temperature in the dark without subculture. After 2 years agar was completely dried but all Asp.species were alive. Water and agar characteristics are important only for growth (vegetative phase) and proliferation (sporulation phase) of moulds.

A8: We grew A. brasiliensis always in SDA - because we had to meet regulations that asked for this medium. Sometimes the ATCC cultures did not sporulate well at first (or the CultiLoops did not either) - and it is true that they do need to be well aerated (grown in petri dish). But we also found that light will make the organism sporulate better. So if we wanted to have good spore production, we would grow it in SDA plate, with the surface upwards and exposed to a good light source (like ambient light in the lab or fluorescent bulb in the incubator). Hope this helps. You will notice that if the plates are stacked, the top ones sill generally produce better sporulation.

A9: I was surprised to know, Aspegillus has a good growth at 37ºC because I always incubated at 25º. I am going to try incubation in screwed tubes, and at the same time I am going to try cryobank.

A10: You can use wide mouth bottle to prepare SDA slants , inoculate the surface, loosely cap the lid incubate for 1-2 weeks to achieve desired sporulating culture then use it. If you have to store this slants cap it tight after the growth and store in the refrigerator.

A11: You can use the following procedure for better storage of Aspergillus sps.
Prepare SDA in Roux bottles which gives more space to allow growth of the fungus.
2) Activate your Kwik Stick with small quantity of (1.5-2.0ml) of sterile distilled water
3) Inoculate the SDA in Roux bottle with the above suspension
4) Incubate at 20-25ºC until it produce spores
5) After that wash the spores with SDB using glass beads 6) Then transfer 1ml of this spore suspension to cryovial and add 1ml of 10% glycerol.
7) Store these cryovials at -60 to -80º C

By this methods you can store Aspergillus up to 1 year.

Fungal inhibition zone

Recently i found one zone of inhibition in SDA plate. it is contaminated with two types of organism, one is white colored colony (Fungus) another colony is looking like bacterial colony center of zone what it may be. what have to do to know i detail.

Tuttnauer Autoclave

I have been using a Tuttnauer autoclave (3870) for one year now, and I was wondering if some of the steps needed in order for it to operate are needed by others with the same style of autoclave.

I currently have to change my water inlet time for different loads. For an 'Unwrapped Instruments' cycle it is at 61 secs, for a liquids cycle I am having to change it to 70 - 72 secs, depending on the volume of liquid involved.

Of course this means that there are times when I have large pools of water in the bottom of the chamber at the end of a cycle and I have to scoop all the liquid out so as not to alter the conditions for the next load.

Is this normal operation for this style of autoclave?

Also, how long do your door gaskets usually last? Mine is beginning to crack and it has only been in operation for one year.

A1: I didn't have to manipulate with manufacture's pre-set conditions for the water inlet time. During validation the sterilization time and drying time were the ones being verified.

It's not normal to have pools of water in the autoclave after the load is done. If it does it might mean that the valves are clogged and water is not returning back to the water reservoir correctly. I have the same autoclave and I found a very nice trick to avoid this.

I placed a hose on the copper coil that is located in the water reservoir and going out to the sink. This copper coil is the one that returns the used water from the chamber back onto the water reservoir, so instead of returning this 'dirty' water back in the water reservoir ....the hose will aid to throw it away (I placed the other end by the sink).

I replace the gasket at least once a year, clean the autoclave with chamber brite (1/2 in the water reservoir, 1/2 in the chamber) once a week, clean all valves (especially the one in the back of the autoclave) once a week and follow manufacturers advice for weekly, monthly, quarterly maintenance needed.

If the gasket is worn out, you can remove it and turn it around (until you get a new one).

A2: I believe that the gaskets wore out pretty fast - about year sounds right, and we had a 4 gallon water chamber that had a drain tube on the bottom, and yes - we did empty it periodically - although I cannot recall if it was daily (believe it was). I have since left the job, and cannot go and check these details.

I know that the water tank was emptied mostly to prevent a formation of biofilm in the chamber, which was otherwise hard to get rid of.

We were also unable to use this autoclave for dry cycles after its first year of use, although it worked fine for moist cycles for over 7 years.

Growth Promotion with Environmental Isolates

Does anyone perform growth promotion with environmental isolates? If so, how do you manage such a program and are there any references for this requirement.

A1: I would argue that as soon as an environmental isolate is put onto growth media, it is no longer an "environmental" isolate. Meaning that its growth characteristics have been altered by sub-culturing onto enriched media. I don't know what added value that brings to the table.

A2: Please suggest me if we can use an archived standard curve for Photometric tests as I am planning to buy an instrument which used archived curve for routine testing.

Please suggest if it is accepted by the regulatory bodies?

A3: I tend to agree. However, the same can be said for type culture organisms. All things being equal, what's more relevant to a firm's media growth promotion regimen?... Demonstrating that a medium can recover (e.g.) an organism isolated from someone's outer ear infection 67 years ago (P. aeruginosa ATCC 9027), or an organism from the firm's own site? I would argue that the use or type culture organisms are completely irrelevant, compared to the use of in-house isolates.

A4: Good point - and the environmental bug has shown de facto the capability to survive/be isolated from an environment of interest.

A5: USP Chapter 1116 does discuss the use of "environmental" isolates recovered by the environmental monitoring personnel during manufacturing for growth promotion.

"In addition, for the Growth Promotion test, representative microflora isolated from the controlled environment or ATCC strain preparations of these isolates may also be used to test media"

"Media Growth Promotion—Procedure that references Growth Promotion under Sterility Tests 71 to demonstrate that media used in the microbiological environmental monitoring program, or in media-fill runs, are capable of supporting growth of indicator microorganisms and of environmental isolates from samples obtained through the monitoring program or their corresponding ATCC strains."

I use recovered organisms, in addition to ATCC strains, regularly for growth promotion of media fills to ensure that contamination by those specific isolates during production would be detectable in the media. Once the organism is isolated and identified, dilutions are performed to ensure counts below 100 CFU. I have only used this process for media in vials, so I don't know about using it for other applications. It's true that the growth characteristics may be altered during multiple sub-cultures onto enriched media, but the organisms were recovered on enriched media (Rodac plates) originally. It is the organisms that can't be recovered using standard laboratory media that we should worry about!

A6: The compendia recommends the use of specified strains for compendial tests as they need to be standardized.

Irrespective of the origin of these stain cultures, these QC organisms have a utility of a long history of use by both media manufacturers and lab users, the ability to source them from national culture collections or prepared commercial inocula derived from those cultures and are GPT requirements for media in general test methods.

Environmental isolates are often mismanaged by the microbiology lab.

A7: I agree with Mr.[name redacted] the use and environmental isolate does not add value to the growth promotion process. For growth promotion I am not aware of any requirement to utilize an environmental isolate.

I would also add the same argument could be said of the use of environmental isolates in disinfectant studies, however it seems to be the industry norm to use them in these studies.

Fumigation in Microbiology lab

I would like to know anybody still following conventional fumigation method of using Potassium permanganate and formaldehyde? How the traces of fumes are checked after fumigation.And Suggest me with best alternative method of disinfection

Kindly refer with guidelines and suppliers if any.

A1: Paraformaldehyde fumigation is infrequently performed on a whole room due to health, environmental, and facility impact concerns. It is more commonly performed in enclosures such as biosafety cabinets (BSCs).

Rooms: I would only recommend fumigating a whole room if BSL3 or BSL4 organisms are utilized. Typically, this work is not in the capability of the facility owner. As such, the work is contracted out to a qualified company. They should present a detailed protocol for the work including, prep, safety communication, dose and dwell, contingency, venting, clean-up, and monitoring. The monitoring is performed with a meter both during and after dwell to ensure target levels are achieved.

I can recommend an excellent company who has extensive experience with paraformaldehyde fumigation.

A2: 70% Isopropyl alcohol and a 5% to 10% Bleach solution are more effective, safer, cheaper and widely applied across industry for microbiology lab cleaning and disinfection.

You can also use commercial products such as SporKlenz and Vesphene.

A3: A good alternative method would be fogging with hydrogen peroxide/peracetic acid products using wet fogging systems like the Hurricane fogger. Coverage and microbial kill can be determined using hydrogen peroxide test strips and Biological indicators for Bacillus stearothermophilus.

A4: I am still fallowing fumigation with Potassium permanganate and formaldehyde but after fumigation i am using ammonia to neutralize formaldehyde fumes and it reduces formaldehyde effect on microbiologist.

A5: Formaldehyde solution 37% (Formalin) to vaporise about 35ml of formalin for each cubic meter of enclosed space.
Potassium permanganate commercial grade.

Fumigate for approximately 24 hours.

De-fumigation

After the stipulated duration , put on the exhaust fan and ensure that no traces of formaldehyde is left.

Use dilute Ammonium solution to absorb the fumes & Clean thoroughly the entire area before the start activities with mild detergents & disinfectants.

After fumigation Settle plates to be exposed to ascertain the Environmental bioload of the area.
The bioload at any given place should not exceed the alert limit.

De-fumigation check:

Take a cotton swab and swab approximately 10 x 10 cms of area near the exhaust grill.

Put the swab into the test tube containing 10mg of chromotropic acid in 5ml of purified water, add 2 to 3 drops of Conc. Sulphuric acid

If violet colour persists it indicates that de-fumigation is not complete.

If violet colour does not persist then de-fumigation is complete.

A6: potassium permanganate & formaldehyde is the best of fumigation.it gives 100% of efficiency.

the traces of the fumes are identified and eliminated by exposure of ammonia solution.

if any fumes are there it gives pinkish colour and this ammonia solution absorbs the traes of fumes.

A6: NSF has approved the use of Cl02 as an alternative for formaldehyde.. no clean up and can easily be done without large up-front expense, let alone without the odor and health problems.

A7: I have been working with the Cl02 for the past 14 months... in our 8 ft. BSC I have demonstrated a 6 Log reduction of atrophaeus spores as per NSF in as little as '2 hrs' with 600ppm vapor concentration and it was without any clean up and a 20 min. scrub. My chemical cost for the Cl02 used in the cabinet was under $15.00, done quickly and to me the best part was without the odor or health issue. Our Lab has done 12 fraction negative cycles with 250, 600 and 1000ppm and have found the use as an alternative to formaldehyde extremely effective.

A8: The real question is why one would fumigate? This is a step only valid if all else fails and you throw your hands up and say, "we have completely lost control". Cleaning and a spray sporicidal application

with 0.52% bleach or a Peracetic Acid & H202 for a minimum of a 5 minute wetted period is a better routine control step. Once you reply on fumigation you set into motion the ability for lack of control and potential safety issues. I'd clean first.

Cell bank testing

I was reviewing 21CFR.610.18 "Cultures" and it states that, “periodic tests shall be performed as often as necessary to verify the integrity of the strain characteristics and freedom from extraneous organisms.” TEM should be an appropriate test to demonstrate freedom from extraneous organisms but I was wondering what tests are being used to verify the integrity of the strain characteristics for a CHO cell line.

A1: I have attached a link to a technical bulletin on "Cell Line Verification Test Recommendations" http://www.atcc.org/Portals/1/Pdf/tb08.pdf

This should help you.

container closure integrity testing

I am looking for information about the size of holes, or breaches of the closure that are being used for controls when the Container Closure Integrity Testing is being performed. I am specifically interested dye ingress studies, but any other information would be extremely useful.

Efficacy guidelines for Veterinary Disinfectant

Please find below is a link to a consultation document for Efficacy data requirements for veterinary disinfectants. Please provide your input:

http://www.apvma.gov.au/consultation/public/vet_disinfectant.php

Neutralization of Azithromycin dihydrate

How we can neutralize Azithromycin dihydrate to recovery microbial growth?

Test for skin flora

As part of our training program for new cleanroom operators everyone is trained in how to properly wash their hands. To show them the need for this, finger dabs are taken before and after washing and after disinfection with an alcohol based hand sanitizer. Normally this results in fewer microorganisms with every washing step and no microorganisms after disinfection.

Recently we found an operator who had Bacillus sp. on his hands after disinfection, repeating the test several times gave similar results.

Does anyone perform this kind of test and what is your experience? Would you let this operator work in a cleanroom (where he will wear gloves of course)? Does anyone know a good sporicidal alternative for the hand sanitizer (and will this work)?

A1: If only one operator had Bacillus spp. on his hands, I would investigate what is going on with him rather than changing your procedure. Any sporicidal agents I know of would be pretty harsh on the skin if used on a regular basis. A discussion with the operator about what could make him different from the other operators might be worthwhile - could he be farming or working on a home garden before going to work? Is there anything he could think of that could make him different?

A2: I suggest you to test the microbiological quality of the alcohol.

And, as you don't explain the method used to washing the hands: 1) Barrs of soap can be contaminated. Liquid soap is the best option. - 2)In addition disposable towers or hot air have to be used to dry the hands.

I suggest you to read: Scott Sutton. "Hand washing - A critical aspect of personal hygiene in pharma". Journal of validation technology - Autumn 2009

A3: Alcohol can also be contaminated - particularly with the spore-forming Bacillus.

A4: Another question to ask is if the particular individual handles biological indicators or handles fomites that may be used by individuals who handle these items.

A5: I also agree with this. Some alcohol manufacturers are involved in the rum industry and so contamination of the alcohol with spores (fungal and bacterial) is very possible.

A6: Does anyone know of a method of depyrogenating plastic containers with hydrogen peroxide? The 250C for 30 min depyrogenation cycle is not applicable.

A7: The is a chemical method using NaOH solution.

A8: Its an odd one but other comments are valid.

Alcohol acts as a very good preservative for Bacillus spores (they will not proliferate but will remain viable in alcohol solution for long periods) also spores would survive in general bactericidal handsoaps containing chlorhexidine digluconate for example.

I think the problem could be the supplier of the hand soap or the alcohol rub and it would be worth tesing for Bacillus spp.

I would not pursue the search for sporicidal skin santitisers anything with that potency would surely be too aggressive to use on the un-gloved hand

Others have highlighted issues with hand disinfection: - One other is that for some individuals rubbing the hands together (as in using soap or alcohol hand sanitisers) removes the dead surface layers of skin from the hand exposing the fresh contaminated skin underneath so skin counts apparently rise. However as Bacillus are not commensals (or unusually transients) as far as I know I think it would be unusual to recover the micro-orgs from the hand.

A9: During 15 years business with hand hygiene, I found often the issue that the skin monitoring after washing and disinfecting shows same or more cfu than before the procedure.
I discussed this issue with several microbiologist from manufacturers and users responsible for disinfection testing. There can be different reasons:

First issue is, that the control of skin with contact plates is not really scientific because agglomerations of bacterial cells will be shown as one cfu on the plate. With the procedure of washing and disinfecting this agglomerations will be destroyed, but if only two cells of a agglomeration survived the process, you will see two cfu on the control plate after washing and disinfecting.

Second issue is the opening of skin pores after washing with warm water - effects the release of germs from the pores

Third possibility - Your soap is contaminated with bacterial spores. A lot of industrial used liquid soaps are not produced under cleanroom or EU Cosmetic GMP conditions. They can be contaminated

Sometimes the paper towels are the source of contamination. The modern recycling paper towels are often not bleached and carry a lot of spores. I know two pharmaceutical companies who identified this as contamination source. Use a white bleached towel.

A contamination via the alcoholic disinfectant is unlikely if it is produced under GMP conditions as a medical device or medicinal product. If only industrial alcohol is used, there is a high risk of contamination with spores.

A10: Any disinfectant in not sterilized until it is treated with sterilization tech. e.g. gamma irradiation, EO or filtration technique.

Before buying any readymade sterilizer check for its sterility and the stability / hold time for regeneration of microorganisms.

Very popular hand disinfectant in clean / aseptic area is filtered 70% (v/v) Iso Propyl Alcohol (IPA) in purified water or water for injection. Filtration of this solution is done by 0.22 micron filter to retain all microbial load. Microbiologist shall do hold time studies on these solutions depending on the environment where it is being used.

A11: Can anyone provide additional information on the use of ethylene oxide to sterilise disinfectants? I would have thought that this would be a process fraught with difficulties since EtO is a surface sterilant and results in glycol and chlrohydrin formation in aqueous systems (the latter is chloride is present).

A12: First of all EO is much more than a surface sterilant. It can penetrate into the most remote locations in a product as long as the product is packaged in permeable packaging. Secondly, EO is NOT used to sterilize liquids! It can, however, be used to sterilize the surfaces of glass vials containing liquids or other packages containing liquids as long as the package is impermeable (i.e., foil). Most liquid products are sterilized with radiation. Also ethylene glycol is no longer a residual that is monitored.

A13: Irradiation and sterile filtration would be used not ETO sterilization.

A14: The PDA Technical Report #7 on depyrogenation has a whole chapter on hydrogen peroxide depyrogenation. There are many times, temperatures and concentrations mentioned. It seems like the most rapid destruction is observed at 100°C with 2.7%. You may want to give the chapter a read.

Bioburden limits for API manufacturing

What do most companies do with regard to setting bioburden limits for non-sterile API intended for sterile product? To be more specific, what are your limits during fermentation, harvesting and purification? For example, some companies go as high as 1000 CFU/mL during harvesting and as low as 10 CFU/100 mL during purification. On a separate note, what is your acceptable percent (%) rate of bioburden excursions during these processes (e.g., annually).

A1: I posted a similar question on Tue, 13-Apr-2010. Our biopharmaceutical pilot plant facility has a history of only ~3% of our submitted samples having any detectable bioburden (per USP <61> filter method), ~95% of those "hits" are less than 5CFU/10ml. We don’t really have an acceptable hit rate, but one would think anything less than 5% cannot be bad given primary recovery and purification steps are non-sterile operations.

I am in the process of evaluating several approaches to determine in-process limits. Thus far I have suggestions ranging from "similar to the bulk drug product prior to filtration" (10CFU/100ml) to "what the process has demonstrated capability to handle" (e.g., >100CFU/10ml). Currently, I am evaluating an approach outlined in PDA Tech. Report #13 regarding nonparametric data to determine limits. Although written for EM data, the approach may also work for in-process samples given the prevalence of "zeros"

and non-normal distribution of the data. Any other suggestions on setting in-process bioburden and endotoxin limits for API bioprocesses would be welcomed.

During cell culture, our expectation is “none detected” given the axenic nature of the culture. Nearly four years of historical data suggests limits of NMT 80CFU/10ml for most primary recovery steps and NMT 10CFU/10ml for purification, but these limits are still a work in-progress as I noted earlier.

Expected rate of environmental monitoring excursions

Hello all. Does you company expect/accept a certain percentage of environmental monitoring excursions per year? If yes, what would that level be and for what areas or processes (e.g., filling, compounding, gowning, laboratory, etc.)?

A1: Isn't the FDA expectation (backed up by the relevant PDA technical report #13) that the Alert Level will be set at the 95th percentile, and the Action Level at the 99th percentile of the previous year's EM observations?

I would hope that your client is using these levels as expectations or has a heck of a good justification as to why they are not. I know that some companies go with some version of the arbitrary (and inconsistent) standards in EU Annex1, PDA Tech Report #13, USP <1116> and some FDA guidance, but this practice (blindly accepting arbitrary numbers) is coming under increased criticism.

A2: I do not believe many people are using this as an Action or Alert Limit. If they did they would be revising their procedures every year. Plus many sites would be 0 CFU/test so what do you do then? Many times the EM observations are so low you would have 1 CFU or 2 CFU as your alert limit.
I have never heard of the FDA dinging people for this. I have heard of EU auditors telling people they need to lower their alert limits based on EM data. They never did it though.

A3: If, for viable particle monitoring, the 95th and 99th percentiles are equivalent numbers of CFUs, is it acceptable to add 1 CFU to the 95th percentile CFU value to set the action level? (Otherwise the alert and action levels would be the same.)

A4: Sorry, maybe I wasn't clear. My question was not related to the actual alert and action levels set by a company, such as 95 and 99% levels based on previous data, but rather, if a company does see an excursion (beyond established levels such as those mentioned above), what would be considered an acceptable or "in-control" percentage of excursions in a year? For example, if a firm observes absolutely no excursions to established EM specs within a year, the annual % excursion rate would be zero. If a firm observes 10 excursions to established specs in a year and the total number of EM tests that were performed in that same year was 100,000, then the annual EM excursion rate would be 0.01%. There are companies that are trending their EM data based on the percentage of total excursions to understand what their process capabilities are and where to look for areas of improvement. Hopefully this clarifies what I am looking for.

A5: Where can i find that doc saying FDA is expecting that the Alert Level will be set at the 95th percentile, and the Action Level at the 99th percentile of the previous year's EM observations?

A6: I would cite the PDA Technical Report #13.

FDAs aseptic processing guide states (in X. Laboratory Controls A. Environmental Monitoring 2. Establishing Levels and a Trending Program): " Microbiological monitoring levels should be established based on the relationship of the sampled location to the operation. The levels should be based on the need to maintain adequate microbiological control throughout the entire sterile manufacturing facility. One should also consider environmental monitoring data from historical databases, media fills, cleanroom qualification, and sanitization studies, in developing monitoring levels. Data from similar operations can also be helpful in setting action and alert levels, especially for a new operation. Environmental monitoring data will provide information on the quality of the manufacturing environment. Each individual sample

result should be evaluated for its significance by comparison to the alert or action levels. Averaging of results can mask unacceptable localized conditions. A result at the alert level urges attention to the approaching action conditions. A result at the action level should prompt a more thorough investigation. Written procedures should be established, detailing data review frequency and actions to be taken. The quality control unit should provide routine oversight of near-term (e.g., daily, weekly, monthly, quarterly) and long-term trends in environmental and personnel monitoring data."

As this text does not explicitly require 95th and 99th percentile, I imagine many will argue they are not required. I will leave it to them to explain how the explicitly stated requirement "One should also consider environmental monitoring data from historical databases, media fills, cleanroom qualification, and sanitization studies, in developing monitoring levels" will be met if not by a recognized method (for example, PDA Tech Report #13).

A7: With the greatest respect I will have to disagree with you. Many companies are not - this is true. However, I will say that it has been my experience that companies with a diligent Quality group and intent on CGMP are not shooting for the minimum they think will prevent them from serious regulatory trouble but rather on a solid program that reflects best current practice (remember that the FDA guidance was published in 2004, the current revision of PDA Tech Report #13 in 2001 - scarcely "hot off the press"). As for ignoring input from regulatory inspectors - a bad idea even if you get away with it for a few years.

If I understand your statement, annual review and evaluation of the EM and sanitization programs is not occurring on an annual basis. This is something I would consider before it is "recommended" by FDA.

You bring up a good point about silly EM levels; 1-10 CFU on a plate is well below the LOQ of the plate count assay. It is of interest to note that the USP has recently put out a revision proposal for <1116> that urges the use of frequency distribution models to determine alert and Action levels in these situations. In this approach, perhaps having recovery on three sampling events in a 10-day period would be the Alert level, rather than caring if there are 2 or 3 CFU on the plate.

A8: Great information. It would be great to hear more on the topic.

All EM Data should be reviewed and graphed for adverse trends. Although I do this weekly. A summary of the on-going trending is captured in an annual EM report with recommendations, summaries, or explanations of how adverse trends are dealt with. It is a good way to promote Preventive Actions.

I am not sure if people vary their action or alert limits based on the annual EM data. Do people change these limits every few years? Action and alert limits, for us, were set from the EM validation runs. The one standard deviation and 2 standard deviation you mentioned were used as the limits. But, these limits have remained constant. I have heard of auditors say EM data in rooms look so good the alert limit should be lower. The FDA does focus on control, if the data remains constant things are in control. If it the data spikes the process may be out of control even below a limit.

I have seen a lot of Limits set as 50% of the Specification for the Alert limit and just below the specification for the Action limit.

A9: Would the approach and expectations be similar to the FDA processing guide in establishing alert and action limits for facilities involved in the manufacture of the nonsterile consumer products? Where is there clear guidance in the development and periodic evaluation of limits in these types of manufacturing environments?

A9: The regime to which Scott refers is, I believe, based on a normal distribution, which is less appropriate for low level "hits"; a Poisson distribution analysis would provide a more defendable statistical model. However, as a starting point, and simple to understand, disseminate and present, the 99%, 95% model is an excellent tool which is used extensively for cleanroom monitoring. I have presented data based on this model to numerous auditors who were all pleased to accept; they are generally pleased to see control

chart based operation of a cleanroom, and are unlikely to delve too deeply into the normal/Poisson discussion.

Yes, good, well run cleanrooms frequently return zero isolations, and thus a single cfu is of course an Alert level response; in fact, for Class A, a single cfu is Action, shut down, throw out the batch...(slight exaggeration for all the purists out there...do an investigation first).
You can't just change procedures/specs because your enviro data is shifting upwards though; the class of room is defined and must be met so the only procedures you could consider changing are the C&S, gowning and training; it is often these that are the cause of an upward trend, and they are the easiest to fix; before diving in and changing all the infrastructure. These "personnel" related procedures should all be reviewed and repeated annually anyway.

What's wrong with a 1 or 2 cfu alert limit? If the site has routinely been free of contamination then any isolation is representative of a change that you should be aware of and respond to before next time it's 10 cfu, and you're actually out of spec; stop work. The whole point of this type of regime is that you can identify and respond to excursions before they are going to stop a shift or lose a batch.

preservative effectiveness test for oral solids

What is the exact method for performing preservative effectiveness test for oral solids.

Now we are following EP mehtod. But in EP or in USP oral solids are not catagorised.

I'm just dissolving 10 grams of the tablets in 100 ml of buffered sodium chloride peptone solution and then i inoculate the required test strains which will be incubated up to 28 days.

We are using potassium sorbate as a preservative.

Can anybody explain me the exact procedure which is adapted for testing effectiveness in oral solids.

A1: preservative effectiveness test is usually for the products that include preservative system. Usually solid oral dose form doesn't have any due to the nature of the product. May be your company wants to reconsider adding preservatives to these products.

The test that should be done for SODs is Microbial Limit Test.

A2: Why would you want to undertake PET for an oral solid? With very few exceptions - such as paracetamol tablets - there does not seem to be a great deal of evidence for such products representing a hazard from microbial contamination (and of course most of them are non-sterile). Many oral tablets are moisture-deficient and therefore unlikely to result in microbial proliferation in situ.

The manipulation of the product you describe would make a PET test very removed from reality, anyway. And potassium sorbate is not exactly a world-shattering preservative.

Or am I missing something?

A3: On the same note I need to test paracetamol tablets for PET but how would I do this. Should I dissolve them in BSCPS + neutralizer but then what inoculums level should I use do I take the dilution into account?

A4: AET is usually applied to aqueous, multiple-use drug products.

Aqueous could be defined as having a water activity > 0.7.

Rarely antimicrobial agents are added to tablets that may be routinely exposed to high humidity environments that may develop mold growth.

A5: No! That's what folks are saying. There has to be a reason or scenario by which PET has relevance to a solid dosage form. Even if there were (maybe it sucks up water such that its Aw exceeds 0.6) testing a form that clearly has to relevance to the product is useless.

If there is a concern, you might look at the cosmetic industry's consideration of face powder preservative testing.

A6: At the risk of incurring scorn from many here who pooh-pooh the need for preservatives in solid dosage forms, indeed, we did routinely add parabens to certain tablet preparations. Acetaminophen based products were notoriously susceptible to contamination with Aspergillus, and I recall a consumer return of a bottle containing pills that were completely black with mold before we instituted this practice. The lesson here was that, despite what water activity you may have in the product when you package it, you never, never can predict what will happen once a consumer gets his hands on it. And that is what the preservatives are there for, after all, to protect the product during its shelf life.

As to conducting the actual challenge, that was fairly straight-forward: we would grind a quantity of the sample, usually 50-100 grams, and add about 5% moisture to it. We would then add inoculum at a 1% level, and do plate counts per standard AET protocols. Neutralizers were used in the diluents and media for plate counts.

A7: I'd like to ask then, based on your message below, since I am one of those who tend to pooh-pooh on these type of requests/needs!

The following: Since you say we can grind the tablets and moisturize the sample with 5% H_2O, this does not reflect actual tablet conditions this being a challenge test, and this does not happen in normal conditions, including the picking up of 5% moisture!

Also I usually had a problem with doing this test for antibiotics in powder form for reconstitution since, in the package insert it asks one to use distilled water to render the sample into a suspension, and then keep it at:

7 days at room temperature or 14 days in the fridge.

The problem I always had with the above was, that to do the PET test one needs to keep the sample for 28 days, which causes a technical problem with the storage conditions:

1 - After 7 days the sample should be discarded as per PI. So we should then rather have an alternative challenge test covering 7 days.

2 - The sample kept in the fridge would be no good as the inocula would be static at best, providing no real challenge.

Although all things considered, the way you propose, we just moisturize and inoculate the tablets and proceed as per the normal test for 28 days, right? Checking after 14 days and then again at 28 days.

A8: Interesting discussion - there has been a lot of debate in the past over conducting an AET on solids. One problem has always been how to inoculate a dry solid with an aqueous inoculum suspension without changing the nature of the material being tested.

How did you determine that 5% moisture (by this I assume 5 mL into 100 g) was within the stability expectations of the product. Actually, I guess the final moisture level was 6% after inoculum. I would be curious as to the justification used (although I imagine a demonstrated fungal contamination issue goes a long way in that regard).

A9: I understand the concept. Sure like to understand the validation to a solid form - of ground material supplement to 6% with water and inoculum.

A10: The choice of 5% was based on studies conducted with unpreserved product: with 6% total moisture added, proliferation of the mold was often observed. It had nothing to do with stability specifications. It may seem that we were being unrealistic, but consider: most medicines are stored in the bathroom, which is probably the most humid environment in the home. Add to that the consumer, who, out of frustration with safety closures or physical limitations, leaves the cap off the product, or worse, leaves the cap off and the cotton plug in (wicking the moisture out of the environment). Under those conditions you may indeed have that level of moisture added inadvertently to the product.

A11: I'll add that the understanding of risk to anhydrous and solid forms is one that has been considered in cosmetics industry for some time - esp for (pressed) powders. Another approach proposed uses membrane overlay and challenge.

Direct contact membrane method for evaluating preservative efficacy in solid cosmetics
International Journal of Cosmetic Science 2007, 12:175 - 183

Microbiological Profile of Selected Cosmetic Products with and without Preservatives after Use
Journal of the Society of Cosmetic Chemists 1972, 23:863-873

A12: Agree with the scenario but limiting it to 6% total because it fails at more seems to demand data that assures moisture uptake in the home does not exceed protocol addition.
Assuming the consumer does not grind up the sample, how did you validate that element?

A13: Why tablets are not blistered?

A14: When you think about your suggestion that the tablets should be packaged in a blister package, it makes perfect sense if the binders of the tablet formulation is able to absorb water from the environment to allow for the development of mold growth. The usage of blister packaging would prevent the tablets from ever being exposed to moisture over the long term. . As an alternative, I have seen companies use small desiccators in tablet bottles to absorb moisture. Granted, it would not be of any help if it is removed from the packaging during usage by the consumer. However, the addition of a preservative to a tablet formulation in most cases would be cheaper than using either blister packaging or adding small desiccators to the bottles of tablets. It would be interesting study to see how much absorbed moisture by the unpreserved tablets was required to cause the development of mold growth.

problems with the absence test of E.coli in antidepressants

I've got problems with the method suitability test of some antidepressants. The products that are showing problems: fluoxetine (prozac) and amitryptyline (tricyclic antidepressant<http://en.wikipedia.org/wiki/Tricyclic_antidepressant>). I know that some antidepressants show antimicrobial activity, but it's hard to find some information about which compound in the molecule inhibit the growth of microorganisms and how to inactivate that compound.

The method suitability for TAMC and TYMC passed by a dilution of 1:1000 for TAMC and 1:100 for TYMC (pour plate), but the absence test of E.coli in 1 gram failed.
E.coli won't grow in TSB in presence of 1 gram product. I've got some validation reports of these products, but they used a 1:1000 (0,01 g) dilution for the absence test of E.coli, which is not in line with the Ph.Eur.

Is there anyone who has experience with testing of antidepressants? What kind of neutralization agent do you use to inactivate the antimicrobial activity of an antidepressant? And which TSB volume do you use for the absence test? And does anybody have some good articles about this item?

A1: I got the same results when testing fluoxetine (prozac). I was not able to find an effective neutralization agent and after several attempts using incerasing concentrations of Tween 80 and Lecithin I reported the

result as being lower than the specified EP value, and declared that the porbability of micobial contamination was low.

A2: Is there no one else who has any experience with method suitability tests of antidepressants? Maybe experience with some other antidepressants?

Lecithin/tween20

I would like to know how you would add the lecithin and tween20 to TSA to contain preservative activity in products.

A1: We use TSA with lecithin&Tween 80 :
- Merck, Code - 1.07324.0500, Tryptic Soy Agar (CASO) with Polysorbate80 and Lecithin;
- Lab M, Code - LAB189, Microbial Content Test Agar
- Acumedia, Code - 7163, Triptic Soy Agar w/Lecithin&Tween 80

PDA Technical Document #13 Fundamentals of an EM Program

Does anyone know how the formula listed in the PDA Technical Document #13, on page 10, "4.6 x (mean CFU)" was derived for determining action levels for non-normal distributions? I understand it is based on the 99th percentile, but does someone have the base formula so I can understand where the "4.6" comes from?

endotoxin standard curve

Does anyone know if it is a requirement to have an endotoxin standard curve from 0.01 to 10 EU/mL? can you have a standard curve above10 EU/mL?

A1: As long as you have appropriately matched standards to the lysate you are using, for a quantitative assay, you can have a larger or in fact smaller standard curve range. You must of course not go below the minimum sensitivity of the kit.
Remember the need to have standards that bracket each log range of the standard curve you use once you are over a 2 log range.
Remember also to have evenly spaced standards along the range.

A2: There is no specifc requirement that states the range that has to be used for a standard curve. The requirement is that there should be a minimum of 3 standards in duplicate with a negative control. So provided you are using a quantitative method with a stated operating range for the particular method/kit you are using e.g. Lonza's KQCL kinetic chromogenic kit has a standard range of 50-0.005, then you can operate in all or any part of that standard range provided:

1. You have doen the initial qualification with an assay that includes the range you are working in. Most users will do the IQ assay for the full range.
2. You have the minimum number of standards as above + the negative control
3. The standard curve meets the criteria required by the BET monographs of a correlation coefficient of >0.98 although personally in this latter case, I would suggest for the kinetic assays, a more stringent internal limit be put on the 'r' value, as a kinetic assay, with data calculated using a log-log transformation, with an 'r' value below 0.99 generally is gives poor results.

A3: If your sample are expected to have more than 10 EU/mL, you can prepare a standard curve above 10 EU/mL.

A4: Actually question raised regarding use of more than 10 EU/ml in standard Curve. I performed several times KTA with 50 EU/ml and that result was acceptable. If you need I will send you. If you use

ENDOSAFE you should be used KTA2 LAL reagent. For Lonza user same reagent can be used which you use in KTA analysis.

suppositories

i want to ask u about microbial count for suppositories how i do it? plz in details after melting it in water bath if i put it in refrigerator to make separation for fatty base are this true or false?

A1: We used to test glycerine suppositories at our factory.

The limits were the same as for oral dosage:

NGT 1000 cfu/g total viable count bacterial and NGT 100 cfu/g fungi and absence of E. Coli per g.

To test we used to crush/squash the supp inside its aluminium foil, then open the one end and squeeze into TSB with Twee 80 and Lecithin.

A2: I have not seen this, but doesn't mean it wont work. Obviously, you should do your Suitability Testing with the same process. I will say, make sure your media is pre-warmed to the required temperature. You must make sure to plate as quickly as possible after placing the suppository in your enrichment media prior to plating, as you do not want to get artificially high counts due to growth in the media. When you do suitability testing, make sure you challenge the window period of earliest plating versus latest plating to show there is no "appreciable difference", on the short end that you get suitable neutralization, and on the high end so you know you aren't spiking your results from normal log growth of the bacteria once in the warmed media.

If Not sure if your intent of the refrigeration is to make for ease of counting on once pour plated, but I would think once you remove the bottle from the fridge, you will have to re-shake anyways.

A3: you can use isopropyl myrisitste to dissolve fatty products instead of phosphate buffers , refer to European pharmacoepia.

A4: I've tried isopropyl myristate for other fatty products to test the suitability, but this plasticizer isn't miscible with TSA and if you add it on dried TSA-plates, it doesn't dry so you can't incubate up-side-down and if you're not careful, the suspension goes over the side. How should I use it? (I still want to try it, because it still makes the best suspensions for fatty products)

A5: Try testing by the filtration method

A6: You Could try pour-plate method. You could dissolve the sample with isopropyl miristate and then pour into a plate 1ml of the sample and approx. 20 ml of melted and cooled to 45ºC agar.

A7: The isopropyl myristate won't mix with the agar (it is not water-soluble) and makes liquid pools within the dried agar.

animal derived peptone versus vegetable derived for use in rinsing solutions?

I'd like to move away from animal derived peptone for use as a rinsing solution but am stymied by the USP <71> formulation of Fluid A that states "peptic digest of animal tissue".

Has anyone had any experience with this type of switch or know where I can find a good comparison of the materials? I've seen a lot of growth promotion comparisons, but since my use is only as a rinse solution I don't think it applies.

A1: There is no compelling reason to eliminate animal-derived microbiological media from laboratory testing. In fact plant-derived may not work as equivalent media to animal-derived media.

Irradiated plant-derived media is the industry standard for media fills where the media contacts production equipment. Lab testing will not have this impact.

A2: Try Hi-veg peptone

A3: Any material derived using animal based media will be subject to additional attention at the marketing authorisation stage, particularly in the EU. The TSE risks associated with the use of such materials will have to be addressed either in the application or via the procedure to gain an EDQM Certificate.

Hydrolysates and endotoxin testing

I was wondering if anyone else has run into an issue when testing samples containing hydrolysates for LAL. We are trying to validate our cell culture media for LAL (Kinetic chromogenic) test and are encountering issues with spike recovery as well as results. The spike recovery is incredibly inconsistent (from negative results to over 200%) The samples have been run several times at 1:10, 1:100 and 1:200 dilutions and often don't pass.

If anyone has any experience with tricky samples like this, please share how you have resolved it / trying to resolve it.

use of sab dex with antibiotics

For MLT testing, how many are using sab dex with antibiotics for Y&M testing?

Can someone explain the rationale why one must use sab dex with antibiotics if bacteria can occur?

Why not just identify if it is a bacteria or not and not count it if you are looking for Y&M on sab dex only?

Why go through the validation of another plate just in case? Or are people just validation sab dex with antibiotics first and not even using sab dex only plates?

A1: you actually answered to your own question. But the logic behind is that some products can have up to a 1000 cfu/g bacteria, but not more than 100cfu/g of yeast or molds or in case of extracts bacterial count can be up to 10000 and be within the limit. in these cases bacteria can grow on Sab Dex and interfere with yeast mold count. Addition of antibiotic will help to reduce bacterial count.

If you company does not have a lot of bacteria recovered from Sab Dex, you don't really have to qualify additional media. and as you said, just ID the growth.

Media Expiration date

I want to know what is the industry practice regarding the use of media. Does your company allows to start an incubation with a media that will expire the same date that incubation starts? Or does the media needs to be within incubation until the end of the incubation period?

A1: We do not allow incubation after expiry. I think that there have been 483 observations on this.

A2: We had a lot of deliberation round this and we've adopted (and documented) the following procedure.

We can use media up to the date of its expiry for subsequent 'routine incubation' from BioMerieux and Oxoid. Both these manufacturers have done validation work and have sent us a document saying that it is

all right to use media on the day it expires for routine incubation. I'm sure other suppliers would do similar, it's just that we've audited these two and most of the media we use is from them!

A3: Our take on this is that the media must be within expiry to the end of incubation. The thinking behind this is that it is during incubation that the media is especially important so as to provide the required balance of nutrients for growth?

A4: The expiration date is just that... meaning do not use after this date. It may be perfectly fine to use, but that is besides the point. Our in-house protocols do not allow to use, read or incubate after that date regardless of the growth promotion properties, etc. that the media may still have. If I had a container that expired that day, I'd reach for another and throw the expired (soon) one away.

A5: To add to [name redacted] comments, if one uses media from any other supplier/ in-house prepared, perform the hold time study of the media with the media plates which are about to expire. If this media supports the recovery , then the media can be used even on the date of the expiry.

A6: Sorry for the delay response. The expiry period of one media is based on its media shelf life validation in which if the validation results shows that the media is worth suitability for its recovery till 30 days(For FTM and SCDM), then u can keep the medias shelf life till 15 days as a safer measure. This will solve your problem. Even on the expiry date also you can use the medium as it is going to be in the study for another maximum of 14 days which shall be already been covered in the shelf life validation study proving its suitability for 30 days.

A7: I (as an BD employee) can underline your statement and give you additional information for the use of our plates. We validate the use/inoculation (and following incubation) of the plates in the same way as you describe it for the BMX and Oxoid plates. Means, you can inoculate the plates at the last day of given shelf-life. Following incubation time was considered when doing the shelf-life validation of the plate. Documentation about this is available. If anyone has specific questions about this, please feel free to contact me!

A8: I cannot see technical data supplied by any manufacturer as to the the suitability of using officially expired media as being acceptable to any auditor. Does not Expired - signify ceased to be valid - or do we have several tacit levels of expiry? Relevant SOPs need to distinguish between Expiry and Use by Date.

As a standard approach I have encountered with all auditors thus far and highlighted by some but not all contributors, media should be within expiry date at the time of test completion.

The key point is that irrespective of what available manufacturer information indicates, the real expiration date is that identified by local GP results and associated validation / stability studies showing that transport, handling, storage and hold duration are consistent with the in-house defined expiry (which naturally should not exceed that stated by the manufacturer or else we open a can of worms with regard to the robustness of any validation that extends the claim for stability beyond that determined through extensive testing by the manufacturer).

1: Surely that is the whole point of growth promotion - if we follow ad hoc the manufacturers tolerance to a stated expiry without local data supporting that claim, why would we need to do GP at all and obviously we are required to do GP.

2: As mentioned by Madupa, an effective approach is to validate a maximum in-house stability period and then set the official local "use by date", by reducing this validated expiry by a time scale equal to the longest potential test incubation period employed. This allow tests be initiated on the final use by date of the media

3: If an incubation period exceeds the validated expiry (in-house) then parallel or retrospective GP must be performed - and while an acceptable approach is not a particularly comfortable position to be in with the possibility that urgent / critical test results may be invalidated. Microbiology gets enough bad press from management without putting ourselves in the dock by our own accord.

Odor testing in Process Water

Need some benchmarking... I assume most people are performing visual appearance testing on process water (colorless, free from visible particulates) but is anyone else testing for odor?

PRESERVATIVE

I am involved in development of a liquid product, in the formulation we have following:

Glycerine -10%v/v
Propylene glycol-40%v/v
Sorbitol solution-40%v/v

My question is do i need to add a preservative system to keep the product safe from microbial attack?

A1: Preservative agents are recommended for multidose products to preserve them from microbial hazard during the patient's use.

A2: If your formulation having 40% of propylene Glycol no need to add preservative, it act as a preservative.

if your formulation having 64% w/v of sugar (sucrose) it is also act as a preservative.

A3: With a 40% Sorbitol Solution and a 40% Propylene Glycol concentration, I suspect that the water activity of the formulation would be low and there might not be the need to include a preservative. I suspect that the formulation below would be self-preserved, but will need to be challenge tested for verification.

A4: To be certain you need to undertake a Preservative Efficacy test. This should be on fresh and aged product - depending on the container-closure moisture absorption might be an issue.

A5: As offered here, these are primarily water activity modulators, tho' you may see some direct efficacy at those levels. As Aw modulators, they'll not reduce counts as much as prevent growth so you'll want clean systems and raws.
Also consider in-use practices - condensation on surface, unmixed water addition will give you localized sugar water.

A6: It would be interesting if you could measure de water activity of this product also.

A7: If microbial attacks occur, add following chemicals as preservatives...

1) Citric Acid --- 0.1 %
2) Potassium sorbate --- 0.1 %
3) Sodium Benzoate --- 0.1 %

A8: Note that potassium sorbate and sodium propionate are GRAS, meaning that they are assumed to be safe at any concentration. Sodium benzoate can be added at a maximum level of 0.1%, according to the CFR.

Another thing to remember is that most of the organic acids used as preservatives are (1) mostly anti-fungal in their spectrum of activity (2) effective only in their acidic form and not as their salts. For example, potassium sorbate added to a product with a pH of 8.0 will exist almost 100% as sorbate and not sorbic acid, which is the species that is active against microbes. pH will dictate how useful these agents are for you. Another possibility is benzyl alcohol. However, if this product is an oral prep, benzyl

alcohol has a discernible taste and an anesthetizing effect on the tongue (like benzocaine) and is therefore likely to be unpalatable to the consumer.

A8: If micro "attacks" happen to that mix, you'd better look hard at raw materials and manufacturing systems.

plate 2 plate variation

We used to perform maximum analysis with duplicate plates, can anyone suggest how much plate to plate variation in count is acceptable? with reference.

A1: Our acceptance criterion is that the difference between logarithms must be less than 0.3.

A2: Which reference is used? Are there regulatory guidelines?

A3: As one previous edition of PMF it suggested the difference should be not more 20%. So it suggested to make 3 plates not only 2, then take the count of two plates and leave any count where variation more 20%.

A3: The USP says 50% difference. If you have limits of 100 CFU the specification is 200 CFU and for cultures to be equivalent they have to be 50% to 200% of each other. I am not sure the chapters. Sorry. Maybe someone knows of the top of their heads. I just read it.

USP <1111> is one chapter that says the 200 CFU Spec.
Duplicate or multiple plates are included.

I tried to find the chapter about duplicate plates it may be within 70% of each other.

A4: No directly.
The difference between logs not greater than 0.3, and the difference by a factor not greater than 2 are almost the same, and:

1) The factor 2 is used as a criterion for GPT of culture media. See USP <61>: "For solid media, growth obtained must not differ by a factor greater than 2 from the calculated value for a standardized inoculum".

2)See chapter USP 33 <1111>: "When an acceptance criterion for microbiological quality is prescribed, it is interpreted as follows: 10^1 cfu: maximum acceptable count = 20; ..."

So I established the same criterion to be applied with duplicates.
Maybe any microbiologist who knows about statistics could tell us if there is any statistical basis to support this.

A5: Are we talking about differences between replicate plates?

I believe the first number referenced refers to acceptance criteria for the microbial enumeration tests. The second is something completely different, the limits for microbial recovery between two treatments (+/- product).

The 70% is described in USP <1227> for acceptance limits on microbial recovery studies (again between two treatment populations).

There are no guidance docs from USP, EMEA, FDA or any other acronym that I am aware of that describe reasonable ranges between two replicate plates. There is a white paper that discusses this topic though. It can be found at http://microbiol.org/white.papers/WP.count.colony.htm.

5 passage culture

Kindly suggest, the culture(S.aureus, P.aeruginosa, B.subtilis A. niger etc.,) which we procuring from ATCC is genetically modified? If the answer is NO, why we are bothering about the number of passage (5) increased will revert the organisms to wild character? plz suggest.

A1: The topic in using the 5 passage rule for positive control or challenge test organisms has been an object of discussion for a long time. From what I remember, the institution of this 5 passage rule was to prevent genetic drift in the phenotypic appearance of microorganisms. For example, I have seen the sporulation of Aspergillus brasiliensis (e.g. Aspergillus niger) change after awhile from using many passages in which white puffs start appearing in the black lawn of spores. By not passing this organism more than 5 times, the appearance of these white puffs do not appear. Furthermore, I have seen the pigments produced by Pseudomonas aeruginosa that has been passed many times not be as robust as those cultures that have not been passed on many occasions.

A2: Bacteria generally have high mutation rate. It takes about 10 generations to ensure at least one mutation. In human time this equals about three and half hours for most bugs. Therefore the further you are from the original culture the more difficult it is to ensure that the resulting organism is the same as your starting culture. For most applications this is irrelevant. However, there are applications (biological assays, reference strains, organisms with peculiar attributes) where it is crucial to work with a strain that is nearly identical to the original strain. If you continuously propagate an ATCC strain you can be assured after some time you will end up with an organism that has physical and biochemical characteristics that are different from the original strain. Phenotypic as well as genotypic studies will be impacted. In addition this makes standardization of methods and comparison of data between labs extremely difficult..... kind of speaking in different tongues.

A3: The point is that you ensure no genetic modification within the 5 passages. In that way you will achieve the same phenotypic characteristics that are supposed to achieve.

A4: USP, ATCC and several other references/sources say "microbial cultures used in the test must not be more than 5 passages removed from the original ATCC cultures".

Why not more than 5 passages, why not 6 or 10? Any studies conducted to support not more than 5 passages? or Is it just '5 for culture passage, like 3 for validation'?

I read article on 'How many passages are too many?' and the references which did not answer my question http://www-fhs.mcmaster.ca/safetyoffice/documents/ATCCHighPassageBulletin6.pdf

Cleaning procedures requalification

In your experience is there any guideline that requires a periodic requalification (as a "must") of Cleaning Procedures in a non-sterile DPs production plant?

Control and uncontrolled area

Is there require control area except Microbial testing room in microbiology lab for API?

If i will keep AC instead of AHU in Media preparation room, Instrument and incubation room, is it valid?

Cleanroom Event

When there is an event within your cleanroom (humidity, pressure or temperature out of spec), how does your facility handle this situation? Does the out of spec need to occur for a certain amount of time before

you take action (for instance you would not react to 1 sec OOS, but you would react to 1 minute OOS)? Do you scrap any in-process product?

A1: When you have an Out of Specification (one second or one minute) you start an investigation in order to search for the root cause of it. Then you have to assess the impact that the OOS could have on your process or products according to its criticality.

Calculation of F0 Value

If both Biological indicator strip and biological indicator ampoule are used in a single load of an autoclave, both having different D and Z value, which one is considered for calculation of F0 value and if both the values are different then how to define the time of the autoclave cycle.

A1: The Kaye Validator can be set to calculate on both. You set up TC groups in the software and plug in the BI certificate values. Also if you want you can run a calculation using the default minimum values for information.

I believe the competitor's version of the Kaye can also do this.

A2: Neither - Fsub0 has defined D and z values. Otherwise, calculate the values for both organisms but do not call them Fsub0.

A3: If I am interpreting you question right, you are using two different styles of biological indicators (ampoules and strips) and trying to figure out which one to base your cycle on. I assume you're in cycle development still.

If this is the case, I think you need to examine why you're using an ampoule and a strip. Ampoule's are appropriate for placing in liquids. Suspending an ampoule in a dry load isn't appropriate as it doesn't take into account steam conditions. You should probably be using either one or the other (ampoule v strip) depending on the application.

If you do need to use two different BIs in a load to develop the cycle, then you should base your cycle on the lower of the two calculated F-bios.

A1: 5.3.1. Your interpretation is correct: 7 days incubation at 20-25C followed by additional 7 days incubation at 30-35C.

4.1.4.1 - Refrigeration simulate you routine process. 2-8C will not kill any bugs, it will only slow them down. However, count of some organisms can increase during refrigeration, therefore, your aseptic system will see higher load of organisms that can possibly end up in your finish product. By simulating you routine you suppose to show that despite refrigeration your system is passing your acceptance criteria for media fill.

I am referring the below points from the guidance document (PI-007-5). Would like the comments from the members.

"5.3.1-It is generally accepted to incubate at 20-25°C for a minimum of 7 days followed immediately, or after a first reading, by incubation at 30-35°C for a total minimum incubation time of 14 days."
This means we need to incubate the media filled vials at 20-25C for 7 days followed by 30-35C for another 7 days. Is this interpretation correct?

2. "4.1.4- If heat is required to dissolve it (medium) then only minimal heat should be used". " 4.2.1.1- If the bulk solution is stored under refrigerated conditions during hold time then this should also be performed for medium"

In 4.1.4, minimal heat is recommended not to reduce the microbial load during compounding. On the contrary, in 4.2.1.2, if the media is refrigerated (if applicable to the routine product load), then are we not reducing the microbial load. Why do we need to simulate the refrigerated conditions during media runs? How do we make sure the refrigerated conditions are not creating a good case?

A2: I you are using overkill sterilization method then, by definition you use a Z value of 10. To calculate the SAL use a D value of 1 regardless of the BI used.

CCI test

This has reference to the Container closure test. Refer the guidance from PI-007-5 as below. "9.1.2 Validation of the closure system by filling the container with sterile growth medium and inserting the container in a broth containing approx. 106 cfu/ml of a suitable micro-organism. The container is removed after submersion for a recognised period of time, disinfected and then incubated for 14 days." Why do we need to incubate the test vials for 14 days? When a bacterial strain is used as challenge strain for this test, any container closure issue must be detected within 3 days. Then why the guidance recommends 14 days period?

Out of Specification in microbiology

i would like some information about the management of a microbiological results out of specification:

* is expected in the microbiological reanalysis?
* if you can reanalyze the sample, must be performed on the original sample or on the reserve sample?
* how many retesting should be performed, for example 2-3 or more analysis?

I would like to know where i might find an official reference document.

A1: this subject was discussed more than once on the forum and it is usually the same answer:

It should be no retest unless you can prove lab or sampling error. If there is a lab error the retest should be done from the same sample. If there is a sampling error, a new sample should be obtained.

A2: You should conduct a retest only if you can prove the results are due to laboratory or sampling error. Then you would invalidate the test and do another test with the same amount of sample. In essence you are saying that the testing process, not the product, is out of spec.

A3: in some circumstances you can not prove that the result was affected by an hidden error (lab or sampling error) if you do not retest the original or a new sample. According to the FDA guidance "investigation OOS test results for pharmaceutical production" released in October 2006, in case of inconclusive investigation, the firm can retest the original or a new sample. This should be done according to a procedure or a protocol in which number of testing and acceptance criteria are predefined. Usually for chemical test a number of 5 - 6 samples are retested so that a statistical evaluation can be done to evaluate that the initial oos result was an outlier. For microbiological testing (i.e. bioburden) this is not appropriate and my question is aimed to understand which is the appropriate number of retesting that is scientifically sound. What is the common practice for retesting of microbiological testing in pharma regulated company?

A4: Generally, retests in micro, esp. for outlier results haven't much use.

See: http://www.silliker.com/html/SCOPE/vol18issue2.php#top

As explained in the above - if the original result had a 1 in 10 chance of detection (say recovery from 1 bug/gram sample in test w/ LOD of 10 bugs/gram), a retest has a one in ten squared chance or recvoery. So you'd have to test 100 samples to arrive statistically at a recovery. That's ideal and we know contamination is usually discontinuous/not homogeneous. In that scenario, you're pretty much certain to get a negative result in retest - and that doesn't justify tossing the 1st result.

Please be very careful with the concept "hidden error (lab or sampling)". Presuming your lab function to be in error is shortsighted. It may appear to excuse one acute concern but effectively pulls the plug on all your data - past and present. If you don't know the error - you don't know if it also provides negative results to positive samples, when it started to effect your results, how to fix it, that whoever designed the program knows what they're doing, etc.

A4: [name redacted] you raise a good point,

As you indicated, the FDA guidance does not include instructions for microbiological retesting precisely because it's allowed only if there is a definitive lab error.

Although chemistry labs can apply the outlier principle it should be used infrequently; a lab that applies it frequently is very suspicious. More importantly the products may be the root cause, not the testing procedures.

A5: Thanks for the reference - that was a good one! You are quite right that the assumption of lab error is one that is increasingly cited as poor GMP by regulatory agencies. If you wish to invalidate the test because the lab messed it up, you must prove that this is the case. Of course the problem with this argument is that if you determine (prove) repeatedly that the lab incompetently performed testing you must eventually question how the lab is trained, led and funded.

I would strongly urge people to pay attention to USP chapter <1117> Best Micro Lab Practices. This draft was published in 2009 (Pharm Forum. 35(4):945-951) and included several significant changes and new sections: - Sample handling (New) - Incubation times (New) - Lab resources (New - includes recommendation on budget and metrics for headcount) - Expanded section on "Training" which includes a discussion on qualified lab leadership.

It is my understanding that this chapter is due to be published as final in Suppl 2 - 2010.

Candida rugosa

Species: Candida rugosa

Macroscopic Morphology: White to cream in color,

Microscopic morphology: wrinkled Branching, short, pseudohyphae

Special Notes: Nosocomial bloodstream pathogen

Does anyone have more information about Candida rugosa?

A1: Hi Jennifer, I am not sure what kind of info you are looking for. Lipases from C. rugosa are perhaps one of the most used enzymes in biotechnology. They have found use pharmaceuticals, production of esters that could not be produced by commonly used methods, in the food industry etc. The organism is also an emerging invasive fungal pathogen. It is of on note because of its resistance to Azoles (Fluconaznole etc.). It is mainly isolated from urine and blood specimens of hospitalized patients. Isolation rates and drug resistance patterns show distinct geographical pattern being higher and exhibiting more resistance to the Azoles in Latin America and Asia.

A2: Candida rugosa is a well known veterinary pathogen that has been increasingly associated with (HAI) hospital-acquired infections within the last 10 years- (nosocomial infections are infections that are a result of treatment in a hospital or nursing home type facility) in burn patients, myelocytic leukemia and catheter-associated infections. The mortality rates associated with these infections can be high, and their frequency appears to be increasing. Antibiotic resistant strains have also recently been reported in geographic areas or clusters. Candida species have emerged as opportunistic pathogens in immunocompromised patients including AIDS patients bone marrow transplant recipients, as well as burn patients. Most Candida rugosa strains are grown in Yeast Mold (YM)Agar or Yeast Mold Broth. The type strain is ATCC 10571 and was isolated from human feces. It produces lipase.

EO residuals

How would you conduct a water extraction for a prolonged contact medical device (>24 h but <30 days). Would a 24 h extraction suffice? I do know that exhaustive methods are necessary for permanent contact devices and that 24 h (worst case scenario) is ascribed to the limited contact devices. But prolonged contact?

Lipase production problem

Please I am working on some isolates that produces lipase. I had one that did very well, so I started my further studies with this isolate. But suddenly the organism stopped producing as there is no longer lipase activity. Please can anybody help me on why I am having the problem. I really need urgent solution. What will I do to revive the lipase secretion in this organism. In any case it is growing well.

A1: This is exactly why we need to store multiple vials of primary isolates so that we can work with passage of stains close to the original. This way we have a source when we losing the phenotype due to continuous propagation in "unnatural" growth conditions. If your stain finds out that it does not need to produce lipase under your growth condition, it will eventually be overwhelmed with low lipase producing bugs and soon you will end up with a population that is lipase deficient. The best way to go about this is get another stain or find out about media/ supplements or growth conditions or stresses that can potentially trigger the expression of lipase gene like providing a lipid (Oleic acid) as the sole carbon source.

A2: Perhaps there have been too many subcultures and/or there were changes in the media.

Try using the original isolate and see what happens.

A3: Lipase in Staph aureus is very short lived on plates. It is a plasmid borne protein and is lost very easy. Once gone it cannot be revived. You should have made frozen cultures and when it is gone start fresh from frozen. The staph. aureus lipase never stuck around for very long. I worked with it for years with the same results. You have to start fresh and have a frozen stock culture that still retains the lipase.

A4: Did you test the identity of the culture again to confirm if you have the same isolate through subcultures or by error you are maintaining a possible contaminant as the lipase producer? Go back to your original isolate and compare [hope you have permed the original isolate!]

Identifying growth when its under the USP limits

My company manufactures a prescription transdermal product. At times we have received results for TAMC and TYMC that are well below the limits set by USP but still indicate a growth. Do we need to have this growth identified if we are well below the limits?

A1: The regulatory expectation is that you have a microbial identification program in place that identifies all organisms recovered in general plate count release testing (TAMC/TYMC). Based on ID, you then make an assessment as to product SISPQ prior to batch disposition taking into account the target end user population, route of administration of the product, etc. See USP <1111>. Note that Tests for Specified Organisms (USP <62>) are not a substitute for evaluating all microorganisms recovered. My impression is the FDA is focusing a lot more on micro in the non-sterile "arena". Sterile manufacturing is kind of easy when you think about it. It's either there, or it isn't. Non-sterile product safety evaluations can be a bit trickier. Good luck.

A2: Assume you determine the isolates are not "objectionables"?

A3: If you get any excursion in future, identification data will help you for further investigation.

A4: If you are not identifying representative microbial colonies from the TAMC and TYMC plates, Phil is correct that you are not able to determine whether an isolated organism from a plate count plate is indeed objectionable or not for the type of finished product being tested. Furthermore, I have seen some regulatory inspectors asked what was the identification of a microbial colony that had been obtained on a TAMC plate for a particular sample.

A5: I think you ask a very sensible question. That is, if the number of microbes recovered is well below the limit, does one need to bother to ID the organisms or at least representative ones. It has always been advisable to (1) find out the source(s) of contamination, even if its does not occur in violative numbers (2) trend the contamination in terms of number and type of microbe. Forty years ago, a species level identification for recovered organisms would have been considered overkill, but with the advent of so many technologies and miniaturized methods for accomplishing this in the past 20 years, it would be difficult to defend NOT identifying recovered organisms to perform an adequate trend analysis.

A6: Ditto [name redacted] comment about determination of whether an organism is objectionable (can grow in the prep, thereby adulterating it, or is a pathogen in terms of the end use of the product).

AOAC COUNTING RULES?

I've used AOAC disinfectants efficacy tests (chapter 6) as ref methods. In the revised AOAC 2010, AOAC Official Method 955.15 and 964.02, section (g.)enumeration of viable bacteria from carriers stated "Use dilutions yielding counts up to 300 for enumeration; plate counts of 0 are to be included in the calculations".

The calculation example for average count (0.1 mL): 115 CFU at 10E3, 15 at 10E4 and 0 at 10E5. The result is 1.17x10E5 CFU/mL . Report as 1.17 x 10E6 CFU/carrier.

I wonder if I have to follow all the AOAC stated or to keep using what the other standard micro. counting rules.

A1: I have not seen AOAC rules for awhile. Most rules state that you only count the plates that have a valid number of organisms on it (30 to 300 sometimes 25 to 250 mold are lower). In your counts the only plate that meets criteria is 115 at 10E3. 15 CFU is not a valid number.

I would count the number as 1.2E5

I think your counts are off by a factor of 10 anyways. 10E6 is 10 fold too high reagardless.

A1: May I make some correction in the previous question.

I've used AOAC disinfectants efficacy tests (chapter 6) as ref methods. In the revised AOAC 2010, AOAC Official Method 955.15 and 964.02, section (g.)enumeration of viable bacteria from carriers stated "Use

dilutions yielding counts up to 300 for enumeration; plate counts of 0 are to be included in the calculations".

AOAC's also stated "The calculation example for average count (0.1 mL): 115 CFU at 10E-3, 15 at 10E-4 and 0 at 10E-5. The result is 1.17x10E5 CFU/mL . Report as 1.17 x 10E6 CFU/carrier".

I wonder if I have to follow all the AOAC stated or to keep using what the other standard micro. counting rules.

A2: There are counting rule in ASTM and Standard Methods for the Examination of Water and Wastewater. The counting rules differentiate between plates counts done by spread or pour plating and those done using standard membrane filters. Spread and pour plating is NMT 300 CFU and membrane filters are NMT 200 or 250 CFU depending on the source of the rule.

UV light effect in dynamic passboxs

UV light is commonly used as sanitizing agent in static passboxes. In dynamic passboxes is there any necessary to use UV light,because dynamic passboxes contains HEPA filters it is kept continuously on.Before keeping any article in the dynamic passboxes sanitize with 70% IPA.it will reduce the bioburden. It is any necessary to use UV light in combination with HEPA filter.

A1: Dynamic pass box (having class A area with 99.97% laminar air flow with UV light)used for the avoid the contamination between the class A area and B area) only - eg (filling area and manufacturing area)

Static passbox the material entry with same class area for eg (transferring the de boxing and visual inspected vials via static passbox to the vial washing area only UV light without laminar air flow).

Environmental monitoring

Why do we need to incubate plates first at 20 - 25°C later followed by 30-35°C for SCDA plates when used for both bacteria and fungi by Active Air sampling method .
For Non sterile area if the fungal colonies grow on the plates first they may spread on to entire space of the medium leaving no place for bacteria to grow.
As fungi can also grow at 30-35°C why cant we incubate first at higher temperature later followed by lower temperature.

A1: It is better to sample the plates in duplicate so that u can incubate one plate at 20-25ºC and other at 30-35ºC. this way u don't have any problem.

A2: 20 - 25°C is the temperature plates are not drying more.Thats why first incubated in 20 - 25°C followed by 30-35°C.

A3: Can you provide Aw data demonstrating that incubation at 20-25C has no significant effect and that permissive Aw is maintained through subsequent 35-37C incubation?

A4: I'm not sure I understand your question. I don't believe that Aw is impacted by temperatures as close as 20-35 degrees C.

A5: Depending on the medium and gill volume, Aw certainly can change through media incubation (see ref below).

Changes in water activity of selected solid culture media throughout incubation
GDG de^ Fernando, O Diaz, M Fernandez, JA . - Food Microbiology, 1992 - Elsevier

The water activities (a w) of selected culture media commonly used in food microbiology have been determined and the effects of the volume of medium poured in Petri dishes, temperature and time of incubation on a w reduction studied. The a w values consistently fell.

A6: Make that depending on medium and fill volume.

Just as with media expiration dates, Aw conditions need to be permissive through the entire incubation period. I know folks successfully use consecutive incubation for 3 days each at the respective temps routinely and have also observed some labs applying the same protocol to establish low Aw's at the end. For the longer incubation periods some mentioned re. EM testing, I'd be esp. concerned.

Urgent Help needed @ use of SDA w/o Antibiotics

I am a bit confused with the new USP 33 <61> Testing of Products - Interpretation of the results section, especially with the reference to use of antibiotics in SDA media. I quote the USP "The total aerobic microbial count (TAMC) is considered to be equal to the number of cfu found using Soyabean -Casein Digest Agar; if colonies of fungi are detected on this medium they are counted as part of TAMC. The total combined yeast and mold count (TYMC) is considered to be equal to the number of cfu found using Sabouraud Dextrose Agar; if the colonies of bacteria are detected on this medium, they are counted as part of TYMC. When the TYMC is EXPECTED to exceed the acceptance criterion due to the bacterial growth, SDA containing antibiotics may be used...."

Now what I am confused about is - during the QC testing of RM/FP, if we are supposed to count all the colonies on that grow on SDA then how does the "When the TYMC is EXPECTED to exceed the acceptance criterion due to the bacterial growth, SDA containing antibiotics may be used...." apply. Wouldn’t use of the antibiotic SDA media inhibit the bacterial growth and give you a false passing result for the TYMC test?

To give you all some more information - I am facing this dilemma with an existing investigation of an incoming RM that is failing the TYMC limit due to bacterial growth. The TAMC is passing due to higher limits than TYMC. The supplier uses the antibiotic SDA and does not see the bacteria in the TYMC and is asking us as to why we don’t use it. And my point is because if we do these would not show up and we would not know that the counts were high in the first place. We don’t want a false negative result! (Note: this is the first time the material has failed for TYMC. Historically we did not have any issues with this material. So we don’t really have an interference of bacteria with the TYMC test here.)

At what point do you decided to add antibiotic to your TYMC test media?

This feels like catch-22!

A1: I am not sure why you are confused as USP <61> is quite specific in its instructions.

The intention is to enumerate bacteria, yeast and mold on the soybean-casein digest plate and report the results as TAMC.

The SDA plate is intended to enumerate yeast and mold using the selectivity of high carbohydrate level and low pH to inhibit bacterial growth. The results are reported as TCYMC.

In some circumstances were there is a high bacterial count or the test specimen contains bacteria that grow on SDA, the fungal enumeration over counts by including bacterial colonies in the enumeration.

To make the SDA more selective for yeast and mold you may incorporate an antibiotic such as chloramphenicol in the medium.

A2: Simply you can calculate your CFU growth in SDA plates if its within the specs which is usually 100 cfu/ml or gm you can pass the material according to the count & history if the cfu were not one of the pharmaceutical reject-able microorganisms .

Current USP EP BP states that even you can multiply by to fold e.i up to 200 cfu/ml or gm

If the cfu are bacteria you can accept it up to 1000 cfu as TAMC and isolate the cfu in SDA by antibiotics if the cfu are mold or yeast you can accept up to 100 cfu as TCYM , if exceeded the limits simply reject the material.

A3: If TYMC of a product is expected to be crossing the specified limit due to the bacterial colonies, then we must consider using the SDA with antibiotics. If the TYMC is not expected to be crossing the specified limits due to bacterial colonies, then we may proceed the test with SDA only. The suitability of the appropriate media must be evaluated during the initial method verification.

A4: Lets make this very simple, When you count for TAMC you use SCDA and for TYMC you use SDA, you need to add antibiotic in case you expect more bacterial bioburden in your sample. TAMC will give you total aerobic microbial count (bacteria and y&m) TYMC will give you only yeast and mold count If you need only bacterial count then subtract TYMC count from TAMC count.

A5: That's exactly the point. They assume that the bacteria growing in SDA may exceed the limit for TYMC, so that's why you can use antibiotic, to avoid this bacteria to grow. In that way you only detect fungi and yeast. I know there's a condition when they say that you must count bacteria and fungi together in SDA, but on the other hand they are giving you a solution, so you really detect bacteria in TSA and fungi and yeast in SDA with antibiotic.

media and sample viscosity

What is the practice or items that can be done to allow a thicker sample when plated to 1) cover the media plate and 2) allow the sample to sink into the media without using a larger dilution?

We have some thicker solutions that flow very slow and take a larger amount time to sink in the prepared media plates.

We truly do not want to go to the next dilution scheme.

A1: I'm not quite sure about the thicker sample you mentioned but included emulsifier such as tween 80 in the diluent may help. I use modified letheen broth/ TSB+ emulsifier as diluent the sample like thick cream can disperse well in TSA.

solid Cfu calculation versus liquid cfu calculation

I have different equations for solids versus liquids for cfu calculation.

It appears that solids actually divide again the sample weight. Why the difference?

Or is this incorrect?

Need to get an understand on this

My understand correct below for liquids and you do not divide again by the sample weight?

If your sample was 2 grams, then your diluents would be 18 mL which would still give you that 1:10 dilution and subsequent sampling of 1 mL from this solution would still result in CFU/gram. (Note: a 1:10 dilution is 1 + 9 [1g of sample + 9 mL diluent]).

If you put 2 grams of sample in 9 mL of diluent, you would then have a volume of 11, not 10, and your dilution would be 2:11 or 1:5.5 so then you would multiply you CFU count by 5.5 instead of 10 to derive CFU/gram.

But for dilution purposes, as an example, if your sample was 2 grams, then your diluents would be 18 mL which would still give you that 1:10 dilution and subsequent sampling of 1 mL from this solution would still result in CFU/gram. (Note: a 1:10 dilution is 1 + 9 [1g of sample + 9 mL diluent]).

If you put 2 grams of sample in 9 mL of diluent, you would then have a volume of 11, not 10, and your dilution would be 2:11 or 1:5.5 so then you would multiply you CFU count by 5.5 instead of 10 to derive CFU/gram.

EM isolates and micro investigation

If one finds an EM isolate on the media plate while doing product testing, can one justify not counting the EM isolate since it is a possible environment contamination?

How does one handle this? And at what frequency does an EM isolate need to be seen to be able to not count the isolate?

Bradyrhizobiaceae in water system - how to get rid of it?

We have an out-of-trend microbial situation with one of our purified water systems in which we are recovering low level of Bradyrhizobiaceae species/Blastobacter denitrificans (<20 cfu/100ml). We have confirmed the lack of dead legs or leakages in the system. We have also performed a passivation of the system, followed by a chemical sanitization with 1% Renalin. However, we still are seeing low level recoveries. Literature indicates that Blastobacter species are freshwater bacteria that form rosette structures by cellular attachment to a common base, which makes me think of biofilm. There were no signs of rouge in the system. I don't have much experience in biofilm and how one would confirm the presence of a biofilm in the system. Any ideas/assistance would be appreciated.

A1: Biofilm, whether formed by a Bradyrhizobiaceae member or a more "conventional" pseudomonad, can be quite difficult to completely kill with chemical sanitization. The sanitant must penetrate into every location biofilm has formed, the toughest of which are in the crevices formed at sealing surfaces between the elastomeric seals and hard pipe surfaces and in some types of non-sanitary valves that can trap water. Also areas such as the dome of storage tanks can also receive minimal contact with the sanitant if there are no sprayballs in the tank returns. In addition to potential coverage deficits, sanitant concentration and contact time are very important. It may simply take increasing the concentration from 1% Renalin (essentially Minncare) to 2% Renalin for substantially longer contact time. The heat from hot water sanitization is quite effective at 65C or above in penetrating into these areas and is a definitive way of killing biofilm, but I presume some incompatible component or lack of access to sufficiently hot water or you would have used it by now.

So if you have no choice but to use chemical sanitants, the hydrogen peroxide + peracetic acid mixtures are about as good as you are going to get (short of using ozone) if given enough contact time to a high enough concentration and thorough exposure to all biofilm-contaminated surfaces. Be certain to flush it through all use point valves and sampling ports in the system to expose these surfaces as well to the sanitant.

A2: Where such type of bacteria is entering to your water system is much more important like ground water, storage tanks, utility. Avoid problem with new replacement. Formaldehyde treatment also more effective.

antimicrobial effectiveness

I am experiencing some trouble in performing AET. After the 14 day sampling of a category 3 product, I have no growth on all the plates. My control plates exhibit growth. Is this a possible scenario? According to the interpretation of results, the product still passes the test as its a higher than 1.0 log reduction, is this correct? I am not treating the product with any inhibitory agents but have been advised to plate using the appropriate media with lecithin and polysorbate, would this be acceptable? please can you advice me on this matter.

A1: Have you validated your AET method? If you see no growth at 14 it could be a good thing means you actually have a good preservative system and that would be an approximate 4-5 log reduction. But if you are killing off microorganisms because you are using media that has not had the proper high neutralizer efficacy and low neutralizer toxicity demonstrated, you result could be artificial. If you haven't validated your method, you are setting yourself up for an observation. Just because you are not adding any neutralizers and so forth does not preclude the need to validate the entire testing process.

A2: If you have microbial contamination in your control plates, it shows that either the media and/or diluents were not sterile or their was improper aseptic technique during the conductance of the test. If you are not using preservative inactivators such as Tween and lecithin, I do not see how you are saying that the product is in compliance with the preservative challenge test criteria. If the same media and diluents where used for the controls and performing the plate counts of the test and no growth was obtained, it shows that the product is inhibiting the growth of microorganisms and the test results are invalid.

A3: Have you demonstrated a suitable recovery method for the product? In other words, residual antimicrobial on the plates may inhibit growth - this must be neutralized.

A4: I just have a few concerns. If preservative inactivators are added would this not defeat the purpose of the test which is to determine the efficacy of the preservative. also, do you add the inactivators to the inoculated sample bottles or can you add this to the media used for plating?

A5: If your controls show appropriate recovery, something is killing bugs or inhibiting recovery in AET. Have you validated recovery from this product?

A6: It should be no retest unless you can prove lab or sampling error. If there is a lab error the retest should be done from the same sample. If there is a sampling error, a new sample should be obtained. And using the appropriate media with lecithin and polysorbate is a Potential Neutralizing Agents and you perfome GPT.

A7: I think that your AET is not validated. You have to use inactivator during test. It should be added in plate but not inoculated bottle because if you add inactivator into bottle, the total preservative will neutralize. As a result you can not incubate again that bottle for another 14 days. Your control is OK. That means residual antimicrobial have in your product or your preservative concentration is high in your product. It may be toxic for your product. So that should be within limit which is specified in formulation guideline. After using inactivator if you do not get high log reduction, your preservation concentration should change.

Validation of kit for identification of gram negative bacteria

We are planning to use a locally manufactured, commercially available kit for the biochemical identification of gram negative isolates recovered during microbiological testing.

We intend to carry out a confirmation exercise for verification of the suitability of the kit.

We currently follow the harmonized method of microbial limit testing for routine purposes.

The kit was introduced into the market prior to the harmonized method of microbial limit testing becoming effective in the EP and the USP.

The use of the kit involves enriching of the isolate in Brain Heart Infusion Broth at 35 – 37°C for 4 hours and subsequently inoculating 50 ul of this broth into each of 12 wells in a sterile plastic kit, each well containing the appropriate reagent in solid form, and incubating the inoculated kit at 35 – 37°C for 24 - 48 hours. Following incubation, reaction of the various biochemicals is noted and results read off against the chart provided.

My question is – since we incubate at a temperature of 30 – 35°C for detection of Salmonella species, P. aeruginosa and E. coli(streaking on MacConkey Agar) as recommended in the harmonized method, would the kit be more appropriately used at the same incubation temperatures for confirmation of the identity of the suspect organisms? If we can demonstrate equivalence of the kit at both the temperature ranges, would this be sufficient justification for using the kit at the temperatures recommended by the harmonized method ?

A1: Please make sure these kits have solid and appropriate data behind them. Are there published data that supports the use of these "locally manufactured" kits?
Playing around with incubation temp with what appears to be a pretty simple set up - 12 substrate based tests - seems pretty unwise. It's unlikely you'll test effectively across the range of isolates - both within a species as well as across diversity of species - to "confirm" (and I'm not sure what that actually means).

A2: You may have to incubate the test kit longer at 30 to 35C the growth rate of E. Coli is maximal at 37C. The kit should work just as well at lower temps. Sometimes in those kits you get the liquids evaporating off. This would happen less at 30C, but may happen more with linger incubation times.
Salmonella and Ps. Aeruginosa grow fine at 37C.

The problem is you are not using the kit per manufacture suggestion so any problems you have would be blamed on the deviation.
The kit is not part of the USP <61> or <62> method so you do not have to follow their incubation temps. Following the manufacturer directions is part of the ID, not USP <61> or <62>, so just say the organism will be ID using this kit per manufacturer's directions.

Really if you validate the method the incubation temp change slightly does not matter until you have a problem and then someone asks, "Are you following the manufacturer directions for the test kit?" and you will "NO" and they will say there is your problem.

A3: I agree with [name redacted] completely. You need to follow the manufacturer's recommendation for the incubation. Perform the validation by challenging the kits using known reference strains. Based on the validation data, the kits may be used. You don't need to compare the manufacturer incubation conditions with the harmonized method. It is acceptable to use the alternate methods provided they are at least equivalent to the compendial methods.

A4: Hold it guys. We have no idea re. current validation of this "locally-supplied, commercially available" identification kit. Running some lab strains to justify incubation at a temp not recommended is hardly validation.

A5: I do not understand what is the issue here, Incubating at 35C-37C vs 30C-35C?

There is an agreeable temperature 35C from both approaches. For validation, can' t the kit be used at 35C and for validation, can't the evaluations be carried out with known ATCC cultures along side the environmental isolates by both methods at 35C..

What can be the validation procedure if this can not be done?

A6: I agree with [name redacted] in that if the Manufacturer's instructions (on which the database of the manufacturer is based and could contain hundreds of thousands of organisms) are to incubate at temp X and the user provides temp Y and "validates" the kit as being suitable at temp Y by virtue of a few ATCC organisms, this is not adequate. In this case, the user is capable of providing what should, more clearly be termed a "verification" that the kit is performing properly. There is a big difference, and so one cannot extrapolate the ID results from a few organisms to use the kit in a manner that is not recommended by the manufacturer. The key questions here is "under what conditions did the manufacturer come up with the reactions that are now in his database?

A7: Please look in the literature for the very extensive data supporting "kits" such as API. API offers 15 ID kits for the bugs it does propose to ID, typically with 20 tests each. We have here an obscure 12 test kit that apparently proposes to do the same thing. As a locally supplied kit, I wonder at its existing validation data at any temp - modifying the manuf's directions would distance one even from that record.

The purpose of an ID kit is to identify virtually all isolates of the bugs it claims not just the USP bugs (that aren't even type species). Rarely are 100% of the isolates of any species consistent in their metabolic capacity. That's why there are typically many single tests in any system - 20 tests in API - even for this, one parses it by larger grouping (e.g. Gram neg nonfermenters).

A8: To validate the kits for use; You must inoculate every well with organisms that will demonstrate a positive reaction in every well. Then you must pick 4 to 6 organism that show the correct ID is obtained. You can meld the two. Do this whole thing 3 times. Every new lot of cards used would have to be qualified for use. Again by inoculating every well with an organism(s) that will demonstrate a positive reaction in every well. This can be done with 3 to 6 cards once.
The manufacture must do the same thing and can tell which organism to use.
They may even sell the organisms to use.

A9: I understand the availability and capabilities of API strips, Crystal ID systems, and other Repid ID systems that are readily available and affordable in this part of the developed world. There are many approaches to identifying isolates, they need not be kits. I am simply giving the benefit of doubt to the alternate source in that region which may be less expensive and readily available. I would think the manufacturer of those kits would have considered basic understanding of microbial identifications and their microbiologists would have given a thought and worked over before commercialization. My suggestion to the initial questioner is to do the comparative studies , publish the validation studies [or get the validation studies from the manufacturer] and then if it is in agreement, use them.

I do not read from Atul below, that he is proposing to modify the kit manufacturer's recommended incubation temperature. He writes he was going to follow the manufacturer's recommended temperature.

A10: identification methods are not part of the harmonized methods, so you are free to use whatever method you like. It does not matter if the system requires BHI, TSB or other media, 15, 35 or 55 °C. As long as it's validated. And as long as you stick to the media and incubation conditions they describe.

If you change the temperature even a little, you cannot use their evaluation tables etc. anymore. Instead you would in deed need to do your own internal validation basically from scratch. Such an internal validation which is accepted by an inspector is a quite laborious deed. I doubt that an inspector would accept a small "equivalency study" (I know I wouldn't accept it). There are too many unknowns: you need to show that under the new conditions not only one but the majority of the strains of a species give good results (you don't want to get a false negative Salmonella ID).

=> just do exactly as written in the manual.

A11: Try this

Cumitech 31
Verification and Validation of Procedures in the Clinical Microbiology Laboratory

A12: I can understand in validating the use of a biochemical kit or system in a laboratory by using the ATCC control organisms that a manufacturer recommends for the Quality Control testing of biochemical cards or kits in order to demonstrate the accuracy of the isolate identification. It is also my understanding that the purpose of repeating the identification for each of the test isolate by using 3 different individuals is to determine how robust is the method for identifying organisms.   When I have conducted validation of these types of identification systems or kits, I had also included several wild-type organisms to see what the kit or system can do with what I call real-world organisms that is not from a culture collection in order to determine how accurate is the database for identifying these organisms. By just using ATCC organisms, you are going to see some variability in the % call for an identification of an isolate or in the actual identification of organisms that might be due to many factors that you do not have control over especially in what type of isolates (e.g. clinical, ATCC, etc) that had been used to generate the database.   In most cases, a laboratory will not be using these biochemical kits or systems to identify know organisms (e.g. ATCC). After the biochemical identification kit or system has been validated for use in your laboratory based upon your own acceptance criteria for an identification call, I do recommend that each lot of cards or kits be QC for accuracy in case they had been damaged during shipment by using the manufacturers recommended QC test organisms that will demonstrate positive and negative test reactions for each well.

If you are going to deviate in how to use a particular identification kit or card from the manufacturers recommended directions, I would suspect that it is very possible to get either an inconclusive or incorrect identification of an isolate because the database is set up based upon how the cards or kits had been inoculated, incubation temperature used and time of incubation. For example, if you accidentally performed the Gram-stain incorrectly by over decolorizing Gram-positive bacilli, you can obtain biochemical identification calls of these isolates for most Gram-negative identification cards or kits. In the end, it comes down to training in how to properly use the biochemical kits or systems. If a person has been improperly trained or deviates from the manufacturers recommeded directions for using an identification kit or system, it will just cause problems in the long run for your laboratory when performing identification of microbial isolates.

A13: In this case, what [name redacted] has said is write.
But one thing can be thought of - You can ask complete validation study performed by the manufacturer in support of your study and demonstrate equivalence of the kit with conventional method!!

Centrifuging in non-sterile bottles

Can anyone tell me if they use non-sterile bottles to harvest cells in a centrifuge. I have never heard of this. I would expect all bottles to be sterile. So if this is common practice I would like to know.

A1: It all depends on what you are going to do with them. (Propagate? purify protein or Nucleic Acids? count, quantitate a particular component?

A2:   We do not use non-sterile bottles to harvest cells when centrifuging them. By using non-sterile bottles, I would be afraid of introducing contamination into the harvested cultures.

A3: You're right - there should be no reason that would make the potential contamination of the cells acceptable. The handling of samples and microorganisms should always be done aseptically, if for no other reason than to avoid confounding the results.

rotation of chemicals for sanitation

What sort of rotation of chemicals are you currently utilizing for sanitation practices in a sterile filling facility? i.e. disinfectant and sterilizers, etc.

A1: If you go through the archives of this forum you will find some answers. Also there was an article published by the moderator of this forum In Cleanroom magazines sometime ago.

In short, use a hydrogen peroxide based sporicidal agent. For day to day use take a pick from Shield Medicare, VAI, Steris and I think Decon Labs. There are not that many companies who make disinfectants for the healthcare industry.

A2: I agree with [name redacted] to the point that rotation of the disinfectants should be based on the type and quantum of microbial data recovered from the clean rooms. If the microbial counts are low and type of hte isolates do not warrant use of sporicide, there is absolutely no logic in including/ rotating a sporicide from the day 1 of the facility qualification. As a precautionary(for increased sterility assurance), one is recommended to use the sporicide on a weekly or fortnightly basis(or at a suitable interval). Other than this, any number of disinfectants (but more than one) is OK as long as the pudding (counts) are low in numbers and acceptable.

Coming to the rotation, one may choose to decide this aspect during the initial facility qualification. Keep the chosen disinfectants rotating at some frequency initially for a period of 7-14 days, evaluate the available data, then relax the frequency of rotation. Based on the data, the rotation can be finalized.

A3: Be somewhat careful about the whole concept of disinfectant "rotation". It used to be cGMP to argue that microorganisms grew resistant to disinfectants in the cleanroom environment and so we needed to rotate disinfectants to combat evolution.

Poppycock (not evolution, the cleanroom risk part). There have been several articles recently that debunk this pharma myth.

Martinez, J. 2009. The Rotation of Disinfectants Principle: True or False? Pharm Technol. 33(2):58 - 71 http://pharmtech.findpharma.com/pharmtech/Regulation+Article/The-Rotation-of -Disinfectants-Principle-True-or-Fa/ArticleStandard/Article/detail/580032?re f=25

Sutton, SVW. 2005. Disinfectant Rotation - A Microbiologist's View. Controlled Environ. 8(7):9-14 http://www.cemag.us/article/disinfectant-rotation-microbiologists-view?page= 0,0

Murtough, S.M. 2001. Biocide Rotation In the Healthcare Setting: Is There a Case for Policy Implementation? J Hosp Infect. 48:1-6

On the regulatory side, the practice of rotating disinfectants is called into question in the text of the current USP <1072>, however, it is encouraged by Annex I of the EU GMPs. I am not aware that the FDA Aseptic Guide addresses the rotation of disinfectants at all.

So, given the scientific evidence I would urge you to qualify your sanitizing agent as effective against organisms in your facility, and on the materials of construction seen there, and then use it in a validated manner. This should be supplemented with periodic "rotation" of a sporicidal agent unless evidence suggests the need to add the sporicide intermittently (again, refer to the current USP <1072>).

Now, having said this there is a chance that the suppression of the normal bioflora of your facility by an effective sanitizing agent may provide opportunity for an inherently resistant microbe to emerge. It must be kept clearly in mind that this is not development of "resistance" by bacteria, but rather a change in the population of microflora in your facility. The sporicidal treatment should keep this under control but you might want to use the opportunity of the annual review of your EM program to re-evaluate the sanitization program. You do not want to start replacing validated sanitization procedures with a "rotation" using unvalidated agents merely because you notice a change in the facility.

A4: As others have identified the rotation of disinfectants often follows the path of one non-oxidising product eg phenol or quat, with a sporicide. Its is also correct to focus on the range of isolates you pick up in your facility and to respond accordingly with appropriate products. Rotation has other advantages (in

addition to the often cited need to prevent the development of resistance) Here are a few secondary but important ones that you should consider when choosing:

A singe non-oxidiser will often not have the spectrum of activity you need to control all microbial species you encounter, mould for example, may be more difficult to control than vegetative bacteria - so it is good to have another option (sporicidal or not) that has a different mode of action or activity.

Having a rotational programme will also give you options if one of your disinfectants leaves more residue than another (favour the one that leaves least residue use the other less frequently)

Rotation also allows you to mitigate against corrosion and other material compatibility issues (use the least corrosive one more often)

Cost (use the cheapest more frequently)

You may have H&S issues or other problems because operators do not like one particular format (pungent smell for example)

One of your disinfectants may have better surfactant properties (eg it cleans and disinfects) so offers options if process residue is an issue.

So you can ring the changes as required and as there are no real guidelines as to the frequency of rotation you can flip at will according to your needs

A5: To [name redacted] point, the rotation of disinfectants seems to be more of an emotional argument than a data-driven one. Since phenolics are not compatible with quats, its been pointed out that since there is always residual left behind on floors walls, etc, the first day of rotation consists of the inactivation of the newly introduced agent by the residue of the old one. When the Agency was big on seeing some evidence of rotation, many companies side-stepped this issue by using LPH (phenolic buffered down to 4.5 with phosphoric acid) and rotated to One -Stroke or another higher pH phenolic so that the active ingredient was compatible, and only pH varied. This served to satisfy inquiries, although I don't know how much was accomplished by this sort of rotation.

Sporicides like Sporecleanse and hypochlorite might be useful if the plant is picking up many spore formers, but it should be kept in mind that to kill endospores, there is a minimum contact time to achieve it (usually ~10 minutes) and most modes of application do not allow this. If this is the case, the use of these agents serves only to calm the nerves of those administering the programs in the plant.

Also, I've seen plants in which the use of a Sporicide is considered the equivalent of "bringing out the big guns" when, in fact, sporicides are no more efficacious against non-spore forming organisms than disinfectants without this added range of activity. In addition, they're invariably corrosive, making surfaces harder to render clean in the future.

A6: Please check this article that I wrote last year. It explains the reality of disinfectant rotation.

Link: http://pharmtech.findpharma.com/pharmtech/Regulation+Article/The-Rotation-of-Disinfectants-Principle-True-or-Fa/ArticleStandard/Article/detail/580032

A7: Please find below is a link to a consultation document for Efficacy data requirements for veterinary disinfectants. Your suggestions/input would be greatly appreciated :

http://www.apvma.gov.au/consultation/public/vet_disinfectant.php

A8: A view in the current guidelines like Annex 1 of the EU GMP Guideline or the Guidance for Industry Sterile Drug Products Produced by Aseptic Processing shows, that the rotation is required but the only

information about the sort of disinfectants is, that one sporicidal should be validated. Furthermore there is no information about the rhythm of rotation.

If you choose the antimicrobial agents, you should have care, that there is no chemical interaction between the different types of disinfectant. In the literature described and from my own experience, a change between aldehyde and amine as antimicrobial agents shows interactions (discolouration of the surfaces. Furthermore, phenolics and QAC can interact.

I got good experiences with a rotation amine based product and a QAC based product (rotation monthly) and a peroxide as sporicidal agent in the background.

Other companies I know used a Amine or a QAC 4 days a week and on Friday a sporicidal (peroxide or chlorine based). So they fulfil the requirement of rotation and the requirement using a sporicidal agent.

A9: The rationale for your rotation should be based on the recoveries from your environmental monitoring program. If your recovery numbers from your aseptic area are very low and continue that way, your disinfectant is working and there is no reason to rotate. if you find spore-formers, rotate in a sporicide on whatever frequency you need it, on on an as-needed bases. see USP 1072 for more information.

A10: The industry standard is to use one or more disinfectants (such as quats or phenolics) as needed to address vegetative bacteria and some fungi. A sporicide is used to address bacterial and fungal spores when environmental trending dictates a need. Therefore, most sound rotational programs have one or more disinfectants of compatible chemistry and a sporicide.
Reference can be found in the Aseptic Processing Guide, Annex I, and USP 32 <1072> as well as several current articles.

A11: I have written many book chapters, papers and technical reports for many years that have been published in the industry and address the myth of rotation of disinfectants. I think the best one was the presentation which I presented at the PDA Micro Meeting in Chicago in 2009 that showed data of a 5 year testing project that conclusively proved that organisms do not develop a resistance to disinfecting agents. I would be glad to forward a copy of the presentation to anyone interested and the publication paper is due to publish very soon. In this testing, organisms were grown, tested and the survivors re-grown and tested again over a 5 year period. In every case, the organisms was destroyed to the same degree as it was destroyed in the first tests. Never did the organism show resistance to the chemical germicide.

By this time most firms have eliminated the rotation of a disinfectant to a disinfectant and have replaced such practice with the routine use of a disinfectant complemented by a the routine use of a sporicide on a less frequent basis than the disinfectant. That rotation exceed the potential efficacy performance of a disinfectant to a disinfectant. Sometimes the general term disinfectant confuses people. Simply, a sanitizer is a product with the ability to destroy low levels of a limited array vegetative cells at low population levels (10eu3) in short contact times that vary per product. A disinfectant provides a broader spectrum of kill over a larger array of vegetative cells at higher populations (10eu6 at normally 10 minutes). An a cold steriliant/sporicide provides the absence of life but is dependent upon contact time. At low levels, below 10eu3 it is effective in short contact times of 5 minutes. But at higher populations (10eu6) such agents may take up to 5 hours.

In reality, the cleaning and disinfection system should be used to address our failures in controlling contamination from entering the classified environment. Once the agent dried it's ability to destroy microorganisms is minimal and such residue presents and even higher level of concern as it may be transferred to an unwanted place and at the same time assist in the deterioration of surfaces. The residual or the use of disinfection as a preventative measure is without technical merit. It has little no preventative characteristics. Control of what enters the environment is the key to success attaining satisfactory environmental conditions. Disinfection is used to address our mistakes in this venue.

70% Isopropyl alcohol

Recently I have had some concern regarding the concentration of the 70% Isopropyl alcohol we use. The company provides a CofA with the product but I would also like to have a method on hand to qc this material. I know normally gas chromatography is the way to go when testing the alcohol content but was wondering if anyone knew of any other methods which can be used?

A1: Option 1: A good test would be to measure the specific gravity and reference against specific gravity tables for various concentrations of Isopropanol.

Option 2: Alternatively measure the refractive index and reference against RI tables for various concentrations of Isopropanol.

Customer audits

I would like to know what is your experience regarding customer audits.
Recently I was audited and all the cupboard were looked in as well as the usual checks.
As for the paper work I have no objection to it, but opening cupboards and drawers? I had a case in the past when auditors checked dates of expiry of the media stored in the fridge, but didn't like the cupboard searching this time!
Any thoughts? Any rules and regulations?

A1: If you are storing dehydrated microbial growth media or biochemical reagents in cupboards and drawers, I see no reason in why the items in these areas cannot be examined. However, before opening a cupboard or drawer, I would think that it would be proper for the person who is conducting the audit to ask a question in what types of items that were being stored in these particular areas of the laboratory before they just go and open up these areas. If it was something that was appropriate for conducting the audit like media or biochemical reagents, I would always ask for permission to randomly open one of these areas for examination. In most cases, I have found that these particular areas were being used to store pipets or other types of items which required no examination. As far as rules or regulations in how an audit should be conducted, I have never seen them, but I think that it should be common sense that proper manners be shown by all parties during the conductance of the audit. It is not worthwhile to start the development of an adverse relationship between all parties due to how an audit was conducted.

A2: I think you need to distinguish what is allowable from a regulatory audit (FDA, etc.)versus a customer audit. Regulators are allowed to see more than customers generally speaking. Perhaps you want to set some "ground rules" before a customer comes to audit or at the beginning of the event. Even further, you may want to incorporate these "rules" into a SOP or guideline and let them know in advance. Also, if it doesn't apply to them, then you can always say that it is proprietary to your company or ask them why they want to know. Sounds to me like they stepped over the edge of appropriate behavior and that could have been part of the test. (They wanted to see how far you would let them go before you said "no.") Some things to think about.

A3: Having been on both sides of this, most recently representing the customer, I have to say that it is not unusual to look in some cupboards, refrigerators, incubators, and other places while conducting an audit. "All" is a bit over the top, unless I peaked into the media refrigerator and found someone's lunch. Then I would tend to look a bit more. Varied experience leads auditors down different paths, some look in the waste bin, others poke around under the sink. All must respect when you let them know a space has confidential information or items in it. But, don't be offended when the report says you wouldn't let them look in that room, they do need to note what they didn't see as well as what they did see.
I would not be concerned unless it seemed malicious.

TSA compare to SCDA

I used TSA and SCDA for environmental monitoring and sometimes, I found fungi and bacteria in both media TSA and SCDA even though I found fungi more in SCDA than TSA.
Did you also count the colony of fungi in TSA? or the colony of bacteria in SCDA?

A1: What reason would you use to exclude the number of fungi on a TSA plate? I would include the fungi in the count on TSA plates if you are reporting the count on TSA as the Total Aerobic Microbial Count.

A2: TSA and SCDAM are the names of the same solid media (Trypticase Soy Agar versus Soybean Casein Digest Agar Medium). Since there is nothing particularly inhibitory in TSA (SCDAM) for fungi, one can expect some crossover of fungi in TSA and SCDAM plates.

If the fungal counts on whatever you are monitoring are being made using Sabouraud's Dextrose Agar (SDA), it is a fair assumption that the fungi growing on the TSA is redundant and need not be counted.

A3: It is common to find some fungal recovery on TSA or the synonymous SCDAM. If one is satisfied that one's monitoring for fungi is adequate, and wishes to make TSA more selective for bacteria against fungi, one can add 10 mg/liter of cycloheximide to the medium. This chemical is a broad spectrum fungicide (Care should be taken in handling it in powdered or solution form as in any fungicide). It will inhibit most fungi.

Should you decide to make this your bacterial recovery media, you will need to validate that this <new> formulation is acceptable in its abilities to recover representative organisms for which it is intended. I find this medium to be very useful to eliminate the problem that you describe, and cycloheximide demonstrates no antibacterial activity - however, since it is a newly introduced entity, validation data must be collected to prove equivalence.

A4: I assume you mean SDA not SCDA? SCDA is the same as TSA.

A5: I use TSA for total bacterial count and SDA for total Mold and Yeast count. If I found the fungi in SDA and TSA, then I just count the colony in SDA only. But if I found the fungi only in TSA and none in SDA then I use fungi count in TSA as total mold and yeast count. Is it correct? and let say, the specification for environmental monitoring in sterile area is <5 for both bacteria and fungi. What if I found 3 fungi in SDA and 7 in TSA. Which one should I count as total mold and yeast count?

A6: Can I add the cycloheximide to the medium and then sterilize them together? What about the antibacterial. I used tetracycline and add them to media (after sterilization) before plating the media, Is there any antibacterial that autoclavable?

A7: if one is monitoring using SDA and TSA, perhaps with a fungal inhibitor, it is important to keep one's terminology straight. Those colonies counted on the TSA should correctly be called Total Aerobic Bacterial Count, and not Microbial Count, which would imply that the data includes all microbes, including fungi.

A8: If you are referring to the harmonized microbial limits test, it is Total Aerobic Microbial Count. All colonies appearing on the TSA plates, fungal or not, are counted.

A9: Per USP <61> it states that, "TAMC is considered to be equal to the number of cfu found using Soybean-Casein Digest Agar; if colonies of fungi are detected on this medium, they are counted as part of TAMC. The TYMC is considered to be equal to the number of cfu found uing Sabouraud Dextrose Agar; if colonies of bacteria are detected on this medium, they are counted as part of TYMC. When the TYMC is expected to exceed the acceptance criterion due to the bacterial growth, SDA containing antibiotics may be used."

This is pretty clear that you need to count all colonies on the respective media. They do provide an option to add antibiotics to your SDA media if you suspect the combined count will exceed your acceptance criteria. In cases like that you may consider using different fungal media such as Rose-Bengal Agar with Supplement C.

fungi contamination in TSA and SCDA

Have you ever found fungi Penicillium contamination in your plate in environmental monitoring in steril area? I found it in both TSA and SCDA I also found it in TSIA media incubate in 20-25. How did you handle this contamination?

A1: You need to go through a great deal of investigation to identify the root cause of this contamination. Will need to check the impact on the products.The products will have to quarantined, during the investigation period and cannot be released until the impact assessment is done and the root cuase identified.Was the contamination in Grade A or B area.It will be more critical, if detected in Grade A area.

A2: I'm still working on the investigation of this contamination I used to check the making process of the media (sterilization cycle) that have this contamination (there's never been issue for this one) review the used of this media in other area monitoring (some of the OOS but some not) check the result of bioburden and sterilization test (especially for sterile product) it's quite safe if the product have post sterilization, but if it's not then we have to do further investigation before releasing the product and yes, sometimes we found it in grade A.

Do you have other suggestion for the investigation? and what did you use to bring the media from lab to the production area? I use plastic container (box shape), but before we use it, we clean it for with alcohol.

The result of bioburden and sterilization test is negative, but the issue is where is this fungi come from. Because the fungi that keep show up still the same one, which is Penicillium. and I wonder this fungi keep exist because it's have been immune to disinfectant that we used daily.

Problems with growth promotion of Staph. aureus

I am having trouble growing Staph. aureus in TSB in closed containers.

The bacteria grow if the containers are vented. This surprises me since the organism, according to litterature, is facultatively anaerobic.

Could there be another reason but the limited access to oxygen that could be the reason of the inhibition?

Does anyone have similar experiences with this organism?

A1: I share your confoundment that SA will not grow in TSB. Is the medium shaken or are we talking screw capped tubes or something like that? It has been my experience that SA actually grows more luxuriantly under microaerophillic/anaerobic conditions, but I've not had an issue getting it to grow under normal aerobic conditions. Again, is your container agitated and to what density are you trying to grow the bug?

A2: We are performing growth promotion on media fill vials post incubation and the vials are not shaken to any extent.

The vials are not screw capped but capped as a normal patient vial.

The whole thing is very strange... The organism grow nicely on our plate controls so it's shouldn't be the stock either

A3: How are the vials stoppered? How long and at what temps are you incubating? TSB is not simply TSA without the agar. It contains glucose and a bit of phosphate buffer to prevent autolysis of organisms that may drop the pH of the media via fermentation. Just an observation - still not an answer to your question.

Are you using a reputable brand of medium? I recall long ago that when I wavered from BBL (now B-D) or Oxoid, my growth promotion studies became problematical.

A4: My experience is Staph aureus does not grow well at 20-25 degree C. If the broth or growth conditions are suboptimal it will bug out

A5: We have found that in some containers the growth of SA is temperature dependent: It will grow at 30-35 degrees Celsius, but not at 20-25 degrees Celsius. In other containers growth is not temperature dependent - we do not know why. I the containers where SA will not grow at 20-25 degrees Celcius the presence of air/no air is not making a difference.

A6: Are you sure that you are using Staphylococcus? If not, it might explain why it grows when vented. Have you confirmed the identification?

Microbial Air Sampler Validation

Does anyone know of a company that offers validation services for Microbial Air Samplers? We need to validate different types of tubing and lengths.

Grow promotion test with Bacteroides vulgatus

I use Bacteroides vulgatus on thioglycollate medium for sterility test.

I have problems to recover the microorganism to the cryovial (the cryovial was prepared with a fresh culture of THIO and 10% glycerol)

Has anyone experience with this strain?

How could i get a fresh culture when i thaw the cryovial? I used to put an alicuot on TSA and incubate it on a anaerobiosis atmosphere. But the strain usually dies.

A1: B. vulgatis will grow on blood agar (e.g., Schaedler's blood agar, or TSA 5% Blood agar) in anaerobic conditions.

Sample Dilutions

A couple of questions I hope you can help answer. My company performs pour plate method to test oral products. We can not always get passing results at 1:10 dilution. Our current practice involves 10g or 10ml sample added to 490mL of diluent resulting in a 1:50 dilution. Further dilutions are occasionally made if needed from this 1:50 (i.e. 50mL of 1:50 added to another 50mL of diluent for a 1:100 dilution). Sensitivity of our test methods were questioned with a recent audit. We add 4mL total to four Petri dishes (1mL each), and then add TSA and SDA melted media to 2 plates each respectively. Do our methods follow the intended USP?

Second question, is Most Probable Number testing recommended by the USP to test yeast and molds? Our audit also recommended we use MPN in lieu of dilutions greater than 1:10. Would you recommend this practice?

A1: In answer to your first question, the sensitivity of the method you describe would be 100 CFU/mL of your product. If your product specs are set there, the criticism might be that even one colony of adventitious contamination would place you in an area in which you're not sure whether its your product or lab contam. If you investigate and decide on a retest to break the tie, therein lies a reason for criticism.

Most oral liquids are preserved with benzoates. USP advises that prior to overcoming inhibition by dilution, various neutralizing media should be used to see if stasis can be overcome by neutralization. I'm assuming that the stasis is caused by the residual of your preservative, unless your formulation itself (perhaps an antibiotic/antifungal prep) is the cause. In that case, there may be no other choice but to use dilution to overcome inhibition. In that case, it is difficult to justify a retest in cases where one colony per replicate appears. I have worked with companies in which they present their work with media containing inhibitors, and in cases of solutions (not suspensions), filtration. The investigator is usually satisfied that they have exhausted all reasonable means of microbial measurement and accepts that dilution is the only way to accomplish it.

Environmental Monitoring Software

If any of you are using a purchased Environmental Monitoring Software, can you share the product name and your experience (good or bad) with the use of that software?

A1: We are using MODA. We have been on the platform since 2006. It is a completely paperless system that has been a tremendous upgrade from the paper based system we employed previously. It has given us the ability to capture more meaningful data and do so more efficiently. Their support and validation teams were very helpful and responsive and truly have nothing bad that I can say about the product.

IV Bag Filling

I have an unusual request for a company that can provide small volume IV bag filling for a clinical trial in Southeast Asia. Bags are 500 mL with volume of about 1000 units per month. If the trial is successful, volume should be about 5000 units per month.

If you have a recommendation, please email me directly - [log in to unmask] Please note that this effort is to meet a humanitarian need, helping to save children.

Cleaning validation

We are a non-sterile OTC manufacturer.(Products are liquids and water soluble gels) Our tanks are cleaned with soap and hot water immediately after the production is complete to remove any product residuals. They are sanitized and rinsed with purified water prior to manufacturing. We use rodac plates and swabs to determine the adequacy of the sanitization. We also test rinse water for removal of sanitizer. However we have not evaluated the complete removal of cleaning agent-soap etc after the cleaning and before sanitization. FDA has asked us to validate this part of the process for the removal of these cleaning agents. What kind of approach necessary and how we should test these.? What kind of soap is used in the industry? I would like some guidance.

A1: The common used cleaning agents are surfactants like Sodium lauryl sulphate etc or other brands which contain these other ingredients. The same can be checked by either flame photometry by calculating the Na concentration.

A2: Its a complicated area

Define all your product contact surfaces (including utensils used during manufacture that come into contact with product) Also be sure you have identified everything, the inside of valves, threaded connections (if you have any) where product can accumulate, dead spots in the pipework, shadowed areas in vessels etc etc. Then make sure you are cleaning them and document the cleaning method.

Consider if your process plant is either dedicated to one product or multi-use

Multi-use more difficult and more crucial to ensure no process residue and particularly no active ingredients pass from one batch to the following batch processes are a little less complex as the risk of active cross contamination is negated and you may get away with visually clean

The type of products used for cleaning vary greatly in strength depending how you clean
Non foaming caustics and acids for automated CIP. Low foaming or foaming mildly alkaline or neutral detergents for manual cleaning

Manual cleaning of systems generally is the focus of regulatory scrutiny as (in theory) manual cleaning is more difficult to validate and therefore is presumed to be less consistent

As far as detergent residue is concerned (not pharma actives) the target is to be able to test down to 10ppm of detergent residue on a product contact surface. You should be able to obtain detergents from a reputable supplier who will provide test methods for you to accurately determine if you have any detergent residue left after cleaning / rinsing.

To be able to test surfaces you need to be able to test rinse water and swabbed surfaces (swab to a to a defined and validated procedure) to show clearance

Test methods vary but often an HPLC method will be provided by your detergent supplier to save you time and expense. The HPLC method often identifies one component of the detergent formula so the assumption being when the testable element can no longer be detected the rest is gone.

A3: To clean manufacturing equipment, the type of cleaning agent to be used is dependent upon the type of product matrix that is left behind. By making an assumption on the composition of your product formulations (e.g. aqueous liquids and gels), I suspect that a detergent containing a nonionic surfactant such as Sodium lauryl sulfate might work best in removing product residues from equipment surfaces. However, this is dependent upon the chemical composition of your product formulations. Sometimes, it is not possible to use only one type of cleaning agent in a manufacturing facility. For validating the removal of the cleaning agent from equipment surfaces, chemical analysis of the concentration for the detergent active ingredient in rinse water can be determined. However, chemical analysis of the detergent active ingredient is specific to the type of cleaning detergent being used. I have seen people start using nonspecific methods such as conductivity, Ion mobility Spectrometry and Total Organic Carbon analysis of either rinse water samples or swab samples to validate cleaning processes. Destin A. LeBlanc has a book on the PDA website called "Cleaning Validation: Practical Compliance Solutions for Pharmaceutical Manufacturing" that you can purchase. I found this book to be very useful in how to validate the removal of cleaning and sanitizing agents.

A4: Sorry forgot to say earlier that non-specific test methods like pH, Conductivity and TOC are also very useful to demonstrate clearance of detergent residue and may be used alone or in conjunction with specific residue test methods.

Also be clear what information rinse water samples and swabs give you. For example rinse water touches almost the entire surface area of your process, its an overview and cannot tell you where residue accumulates but that you have some somewhere. If rinse water quality in and out of your plant does not change do not accept this as a signal that all is well, it may just mean the residue is poorly soluble. Swabs sample just a tiny spot in one area (so important to design a good swabbing plan) to get a wider picture of what is going on in your process from one end to the other

You may need to use both methods to get the full picture.

A5: There are published articles related to determining acceptable residue limits in cleaning validation. A common method for a drug active is to use a calculation based on the minimum daily dose of that active into the maximum daily dose of the subsequent produced product. However, for cleaners, some toxicity measurement is used in place of the minimum daily dose (e.g. acceptable daily intake). Please let me know if you would like some of these references.

There are different types of cleaning agents available and more than one can be effective in removing your soil. Three categories are used in the pharmaceutical industry: commodity chemicals, organic solvents, and formulated chemistries. From these three, formulated chemistries offer the advantage of several cleaning mechanisms to achieve the best performance (e.g. surfactancy, emulsification, wetting, sequestering, etc).

Selecting the right cleaning formulation may require technical support and cleaning studies. This is why it is important to consider the following tips to determine the cleaner that best suit your specific needs:

1. Broad-spectrum = a single cleaner capable of removing several soils.
2. Rinsability of the cleaner = a cleaning agent that is easily rinsed can reduce time and cost.
3. Consistency and stability = supplier must be able to provide batch to batch consistency, lot traceability, CoA's, stability information, among others.
4. Safety and toxicity = cleaner must be safe to handle by trained personnel and suitable for the application. Also toxicity of the product is important to determine acceptance criteria of residues left behind on the equipment.
5. Environmentally friendly = product should meet local environmental regulations and should not contain hazardous pollutants.
6. Long-term availability = cleaner must be available for a long time without changing formula. This will assure the validated state of your cleaning process.
7. Technical services = supplier should be able to support product application, provide guidance on selecting cleaning parameters, and facilitate documentation relevant to the cleaning validation process like analytical methods for residual quantification of the cleaner, technical data sheets, toxicity data, among others.

A6: TOC measurements are the most common measurement to show residues have been removed. One approach could be to take TOC samples throughout the validation to determine when the TOC levels reach the desired concentration. For example this may take a final rinse of two minutes. Then your validated rinse would be at least two minutes (I would use two and a half to exceed what is needed).

You could also do spike studies in the lab to show effectiveness.

aseptic process validation media transport

Could someone please tell me if tryptic soy broth used in aseptic process validation would be considered biohazardous waste in terms of transport to another facility? Could you please refer to documentation.

A1: Not unless it is inoculated. It would in the US be considered chemical. Solution subject to federal and state labeling laws and chemical manifesting.

A2: No. Please see related guidance http://www.cdc.gov/od/ohs/biosfty/shipregs.htm
http://www.cdc.gov/od/ohs/biosfty/shipregs.htm

Check your local state regulations.

incubator versus show case

I used incubator30-35C for pre incubation and incubation of TSA media, and show case 20-25C for pre incubation and incubation media SDA. and I found more condensation issue in SDA than TSA. and again the fungi contamination in SDA more. Is it related to the use of show case to store the media?

Pyrogen-Free Serological Pipets

Can anyone recommend a source in the USA for pyrogen-free (5mL) serological pipets? I have only been able to find serological pipets that are certified to 20 Endotoxin units per device, which would not be ideal for use with LAL when we are running assays to 0.005 EU/mL.

A1: I purchase them from Charles River Endosafe.

A2: 20 EU/device is the compendial endotoxin limit on all devices (not in contact with the cerebal-spinal fluid). Therefore this is the limit manufactures are going to be tested too. However the nature of disposable plastic pipets and they way they are manufactured makes them a very small risk for endotoxin contamination. I know that we use sterile, non-pyrogenic (tested to 20EU/device) pipets (VWR) and never see issues even when testing down to 0.005 EU/mL.

A3: VWR has nonpyrogenic, borosilicate glass serologic pipets in various volumes (including 5mL). Their website is https://www.vwrsp.com/index.cgi.

A4: Although manufactures have made great advances in producing high quality disposable plastics for use in the laboratory, the use of any material in the endotoxin assay that is not certified pyrogen free or certified to at least the sensitivity of the lysate could pose problems. How can you guarantee an OOS result was not caused by an article used in an assay that has a potential endotoxin level higher than the specification of the sample being tested? Glass serological pipettes that have been rendered pyrogen free by a validated dry heat method are your safest bet. If you do not have your own dry heat oven that is validated for the process, there are pipettes available that have been rendered pyrogen free by validated methods. If you decided to validate your own oven run make sure you remove the cotton plugs from the pipettes. I have yet to find unplugged 5mL glass pipettes.

A5: Thanks for your quick response. However, when I looked in to the VWR individually wrapped, sterile, non-pyrogenic serological pipets I was issued a certification that claimed that they were tested to 20EU per device, and that they average about 3 EU per device. While I cannot claim to understand how they test them, 3 EU within the 5 mL pipet would be about 0.6 EU per mL, on average. If the endotoxin is on both the outside and inside of the pipet, we are still estimating about 0.3 EU per mL internally in the device.

I am curious to find out how other labs deal with this issue. Do most of you test with this risk? Do you QC your pipets to 0.005 EU/mL in house (if so, how would you do so with that volume)? Or do you simply depyrogenate your own pipets?

A6: The other important aspect to remember is that the contact time of the water/test solution/etc within the pipet is not nearly enough to extract measurable amounts of endotoxin from the plastic given endotoxins affinity to bind to plastic. The extraction performed on the pipet is no doubt done with a lengthy hold time (generally one hour), versus the few seconds of contact during testing.

A7: Charles River Endosafe supplies 1 mL and 5 mL pyrogen free glass (borosilicate) pipettes.

A8: [name redacted - if you are still concerned with the low limits, you can get your own depyrogenation oven, and depyrogenate your own glass pipettes (you'd need to check the temperature required - but I believe we used to use about 375 C for maybe 1/2-2 hours - I am not sure any longer).

We also worked with very low limits, and never had any problems using plastic pyrogen-free pipettes. When I used to do the LAL test, the pipettes were not even labeled as pyrogen-free. But I understand as someone else has already stated here, that the molding process virtually eliminates all endotoxin.

There can be a chemical contamination issue though - I recall running into a batch of plastic pipettes that gave us false positive results. The manufacturer was aware of the problem and warned us about this issue eventually. Not sure if they knew what caused this false positive reaction. But in my 30+ years it happened only once, and in the very early years of LAL development.

A9: I would be careful. Utilization of any disposable commodity that is not certified pyrogen free to the sensitivity of the lysate could result in false positives. Although vendors guarantee tested to 20 EU/ device, we have seen some of our consumables occasionally spike when testing at 0.005 EU/mL. As a result we screen our consumables commodities that do not carry a low enough endotoxin free limit by testing them. Per USP you need to verify that your commodities do not interfere with the test.

Quality Management System of the Laboratory

I would like to ask a question to quality personnel within the laboratories. I have seen many laboratories supplying the pharmaceutical industry, some ISO 17025 accredited and some not. In my opinion, the ISO 17025 accredited companies are by far more customer oriented and appear to be more quality focused. Do you feel that laboratories within pharmaceutical companies or laboratories supplying the pharmaceutical industry could benefit from complying with the ISO 17025 standard or is following the requirements of cGMP good enough?

A1: I think you should read this article by Scott Sutton in
:

Pharmaceutical Microbiology Forum Newsletter - May 2009, Vol. 15 (5) Page 2 of 12

Is This Contract Microbiology Laboratory Qualified to Perform Testing?

Author: Scott Sutton writes:

The absence of this accreditation is not evidence of poor recordkeeping practices, or a poor Quality system, but it makes the audit more difficult. Let me repeat that point - many excellent labs have not pursued ISO 17025 accreditation and its absence should not be construed as disqualifying that lab from consideration.

A2: While I do see value in accreditation, if the company is not quality and customer focused going into the process, they will not be after. Often enough I have experienced working with (at least trying to) companies that have some sort of certification or accreditation that is only a marketing tool for them. Some laboratories would benefit, some would not. It depends primarily on having management that is committed to quality and customer satisfaction.

Basic Degree in Microbiology

I recently faced an interview in multinational pharmaceutical company in my country for the position of Manager, Microbiology. In the circulation they demand Master of Science in Microbiology with 5 to 7 years experience. I applied and they called for interview. The interview board asked me few question regarding managerial activities and I replied everything. The board pleased on my reply. But they tell me that you can not study in B. Sc in Microbiology. So its a lack for this position and Bachelor degree in Microbiology is basic degree. I completed B. Sc. in Biology and M. Sc. in Microbiology. Now my question- is it lack for that position ? Do I deserve that position ? What is the international regulation for Manager, Microbiology position in multinational pharmaceutical company ?

A1: As I understand your note - they rejected you because your underfrad was bio rather than micro. Can you ask them the skills/experience (mycology, etc) they see as acquired in completing a bachelor's degree that they see as critical - and show them (if possible) you have acquired these skills?

A2: As far as I know there is not specific regulation at least in the US. However, we have summarized requirements for different levels of expertise in pharmaceutical microbiology in a recent publication. Here is the reference:

Claudio Denoya and Jennifer Reyes. (2009). "Pharmaceutical Microbiology and Rapid Microbiological Methods: Reform Needed in the Microbiology Curricula by Universities to Match the Skill Set Required in the Quality Control Labs". RMUG Rapid MicroUsers Group Newsletter, 9(1):1-11.

A3: If you have M. Sc. in Microbiology and 5 to 7 years of experience in microbiology along with managerial skills, you seem to be qualified for the position.

A4: I'm the head of the microbiological department in a Pharmaceutical enterprise in spain. I have a degree in Biological sciences, specialized in microbiology. This is enough together with a demonstrated experience to get a job as head of microbiology. I got my current job 2.5 years ago.

A4: I have to mention that the revision of USP <1117> Best Micro Lab Practices also addresses this point with some significant changes in expectations for lab heads.

A5: I have a BS Degree in Microbiology and a PhD in Food Microbiology.
For me it depends on the school you went to get your degrees.
The schools I went to taught a lot of basic Microbiology that you may not be able to learn over time due to lack of qualified teachers. At one time Microbiology was one of the hardest majors to achieve. You had to take the hardest Organic chemistry classes, biochemistry, physical chemistry, and many lab classes. That has changed at many universities and they now are focusing on molecular biology.
Looking at your transcripts and the classes you have had I want to see that you had a lot of chemistry, biochemistry, organic chemistry, Physical chemistry, calculus, statistics, and physics. Hans Christian Gram was an organic chemist. Pasteur discovered stereo isomers in chemicals.
What was your GPA in those very tough classes. Your references would be important.
It has been universal that biology majors want to go to medical school so they major in biology to up there GPA. Biology has been a weaker major in basic sciences. I deal with many young college graduates and people can learn if taught the right way. So part of it is who taught you the right way. Are you a lifelong learner. Have you read the USP chapters? FDA guidance documents?

Keep your chin up and ask yourself what extra training can I get so this does not happen again. I have gotten a professional certificate even after my PHD to stay competitive. Never turn down free education.

mold action level in EM

Anyone know if it is still a requirement to set "action level" for mold found in EM? I went to PDA training and they said that FDA only required to set action level based on count only? and mold should be considered as a type of bacteria? any thoughts would be helpful.

Number of Total Air Particle sites required per room from the ISO system

In the ISO system you figure out the total number of sites required, for total air particle counts, per room by finding the area of the room in square meters. The required number of Total Air Particle sites required for that room would be the square root of the area (square meters).
For a room that is 28 square meters the number of sites would be 5.3 particle sites.
Does anyone know if you can round the number down or do you always round upward to the next integer? So would the number of sites be 5 or 6.

A1: It is better to round off the figure upward to next integer, so sufficient number of samples can be taken, which ensures that you have covered all area representatively and no area is left un attended.

A2: The ISO says "rounded up to a whole number”.

validation of cryopreserved strains

We preserve our ATCC strains after reconstitution through a cryopreservation system. How can I validate the expiration date of the cryopreserved strains, because the manufacturer says "If the lyophilized microorganism is cryopreserved before its expiration date, the expiration date of the cryopreserved product will be as determined by your cryopreservation procedure. Microorganisms that have been cryopreserved are not covered by warranty and cannot be replaced"

Is there a method or a International standard for this? which one?

A1: I have not heard of a standard that can be used to set the expiration date for cryopreserved cultures. I would think that you would run periodic viability studies of each set of cryopreserved cultures to determine if they are still viable at different time points.

A2: Our internal SOP for stock culture maintenance/storage used to state 5 years expiration for cryopreserved cultures similar to yours. However, we found that at the end of five years, some cultures were showing variable viability. As such we revised the SOP and now use 4 years expiration for such cultures, without issue. I think you definitely need to establish an expiration date and have it documented in an official way; SOP, method, etc., to be safe.

USP <81>

I have a few questions regarding USP <81>.

I was wondering if anyone knows where I can find the specifics for extracting a cream vs. an ointment in USP <81>. I have searched but do not seem to find the section that specifically mentions how to extract the sample.

We are looking to purchase a new vacuum oven for drying out the USP standards; does anyone know a model that they find works well for drying out the antibiotic standards? We are looking at different options.

Should the sample be weighted out specifically prior to extraction to meet the same final diluent as the reference standard or is creating the dilution after extraction to meet the final diluent as the reference standard is acceptable?

Sterility isolator problems

I am having issues with sterility isolators to test products. Decontamination cycle won't pass. I am recieving an alarm "preconditioning time exceeded"

A1: Did you try to regenerate the dehumidifier (if present)?

A2: From my (dim) memories of systems, this would indicate you're not achieving your preconditioning parameters- most likely humidity and/or temperature. Have you done distribution on your isolator, checking how well air is distributed in the chamber (also how fast and how well the dry, heated air and the sterilant are distributed)? Check with your sterilizer manufacturer for assistance on this alarm?

A3: This sounds like you may have a Skan isolator. If so, there is a pre-condition run time error of 5400 seconds. The alarm that you got would occur at this point. Causes for this would include RH sensor malfunction, RH set point too low, humid air is leaking into the enclosure somewhere, or the compressed air is not dry enough. Per Mike's comment, I would run this by the vendor to get it resolved.

A4: Do you know what's the humidity is inside the isolator? In your cycle parameters, there is a 'Dehumidification time', there is min and max, try increasing those, basically your Decontamination unit is trying to dehumidify the isolator before it moves to the next stage in the cycle. You can watch the humidity level while the unit is dehumidifying, if the isolator is not placed in a controlled area (some are and some

are not) then you might see changes in the humidity, you might have to adjust this parameter as needed. That should take care of it.

A4: If your isolator is decontaminated by a Steris generator, then there are a couple of things you should check:
1/ Desiccation capacity: if the dryer cartridge/tank has not been regenerated, then you won't be able to reach the humidity level targeted.
2/ The temperature of the preheater and/or the heater cannot be reached. Check the RTD probes and the heater bands.

A5: This may mean that your vaporization plate is not reaching proper temperature.

Environmental monitoring of non sterile area

Please give me some information about sampling points for environmental monitoring of non sterile area. In case of sterile area we prepare a monitoring program where we indicate all places of sampling. What should we do in case of non-sterile area? For example due to our state regulation we must control different point every time and in this case we can't indicate the sampling points in the monitoring program. What should we do according ISO regulation?

A1:
1. you must divide ur areas to zones then you determine sampling points in each zone
2.you have to put a sampling plan and time table
3. take 12 EM results weekly this include personnel, surfaces, and air
4.build up by statistical data alert and action limit
5.Then minimize the no of sample in ur sop and confirm it.
6. then frequency of EM monthly.

Raw milk

Why are people insisting on having raw milk?

I milked cows as a lad in New Zealand and one of my jobs was shoving manure from the milking shed.

Attached is the website of the food-borne disease lawyer Clark Mailer with a discuss of the issue:

http://www.foodpoisonjournal.com/2010/05/articles/foodborne-illness-outbreaks/raw-milk-an-issue-of-safety-or-freedom/index.html

A1: I am going to preface my response by first admitting that I am in no way an expert on this subject. I also am not a milk connoisseur - I like to walk into my nearby grocery store and buy a gallon of Whole Milk (of course I scan all the containers to choose one with the most distant expiration date available). My knowledge on this subject most likely doesn't expand beyond the first page of Google search results you would receive from an online query. Also, I like knowing that my milk has been pasteurized, and I am not at all going to advocate drinking raw milk.

With that disclaimer out of the way:

I'll first have to nibble at the question: "Why are people insisting on having raw milk?" I can see this question being asked two different ways. You could be asking "Why would anyone want to drink raw milk?" or "Why do people want to have the right to drink raw milk?"

For the first version of the question, there are a variety of answers. Some people like the taste. Maybe some people like its usefulness in making home cheese or butter, or enjoy crème fraiche. Some people

are opposed to the antibiotics and hormones forced into dairy cattle to make them milk-producing machines, and think that raw milk from free-roaming cattle is more tolerable. In America at least, there is a huge movement (for some bizarre reason or another) in a Paleolithic approach to health. See the movements of barefoot running, or the Paleolithic diet. Non-scientists (and I'm sure some scientists as well) put a large amount of faith in the idea that homo sapiens evolved to eat certain things and that our "tampering" with the natural way of things makes us more unhealthy. I have heard the argument that raw milk contains many vitamins and minerals that are destroyed through pasteurization.

The other version of the question is political in nature. Helmets on motorcyclists, seatbelts and airbags for car drivers, and the FDA cracking down on the sale of raw milk by Joe the farmer. All are cases of the government interfering with someone's rights for the sake of their own safety. "Should" the government have the right to do that to an individual? You are bringing up a subject which is very far-reaching.

Ingesting raw milk has some risks associated with it. I think most private sellers would prefer that it be unregulated so they can fill the mason jar with it and sell it to their neighbor, and they assume that any potential buyer of raw milk would know the risks involved. Then again, even Drano has to write "do not drink" on the bottle because some idiot thought that it would be a good idea. Maybe one could argue that raw milk should be allowed to be sold, so long that it is clearly advertised as raw milk. I think it would be a bit odd to see a mason jar of raw milk with a "black box" label attached!

I'm not sure if I answered the question, or if it was even the type of question that has an answer [name redacted]!

A2: You must have had an interesting youth, most folks have only seen milking operations in a movie or on TV. It is amazing how removed we have become from the source of our food.

I see a growing concern in our society with this "distance" from our farms. This is reflected in some of our eating trends such as an increasing preference for organic products and "whole" foods (unprocessed).

Proponents of raw milk consumption are a segment of the "whole food" advocacy. These people argue that raw, whole, unprocessed foods contribute to better health and nutrition than their "processed" counterparts. The primary cited differences between raw and pasteurized milk include natural enzymes and normal flora. However, the concern about raw milk is not from the presence or absence of these components but from the potential for pathogenic contamination.

Many of our health concerns in agriculture stem from the development of factory farms and poor practices that contributed to an increase in food contamination. These include continuous milking, use of manufactured feeds, larger herd sizes, and increased concentration of livestock per land area. To put it in GMP terms, our farms were filthy and building-in quality at the end of the process (via pasteurization). So, it seems that we have created the epidemic ourselves by not keeping quality first throughout the process.

I happen to live in a farming community and raise some of my family's food on our small farm. I have visited many farms employing diverse practices and having varied control processes to ensure the quality of their products. Although all of these farms meet minimum federal and statutory standards, some of them NEED to have their milk pasteurized while others employ rigorous sanitation methods (AKA: a quality system) far exceeding the minimum.

From a risk-based approach, I think you cannot have it both ways. If minimum standards allow farmers to have a dirty pre-milking process and then clean the product at the end of the line, then you cannot allow raw milk to be sold. If however, rigorous standards are implemented, then raw milk risks can be mitigated and the sale should be permitted. This has been successfully done in California.

A3: Unpasteurized milk has many health benefits to it that are not found after heating the milk. You can google raw milk benefits, or here is one site that explains a little.
http://www.thedailygreen.com/living-green/blogs/organic-parenting/raw-milk-55061801

A4: Let me add that drinking "raw milk" is not an option in some underdeveloped countries, it is the rule. I grew up in my grandparents' farm in El Salvador, and from what I recall, we used to drink fresh, 'out-of-the-cow' milk. My cousins and I grew up very healthy and without any of the diseases that many of our children nowadays suffer. I guess this helped to boost our immune system to some degree.

Even to this day, I look forward to going back every summer just to drink "raw milk".

So, I agree with you in that we cannot have it both ways. If we permit farmers to have poor sanitation practices from the beginning, then we will end up with contaminated milk. Quality must be built into the product from the beginning. By raising the quality standards, we have a better chance of getting a cleaner end-product.

A5: Cindy - not much there but anecdote and observation that content of some vitamins. Can you provide any technical references?

A6: Don't have the references at my fingertips, but I've read some before. Don't have time to google for them now, but I'm sure you could find some w patience. Sorry I don't have the time.

Gloving & gowning

Question on what everyone's practice is on gloving and gowning for clean room using 2 pair of gloves and sterile sleeves.

You put the first pair on down everything (boots, bonnet and boots)

a) Take the first pair of gloves pull then out from under the gown sleeve and place then over the gown sleeve

b) Put a sterile sleeve of arms

c) Put the second pair of gloves over the sterile sleeve and first pair of gloves and enter the clean room

In short 1 pair of gloves over the gown and the second pair of gloves are over the sterile sleeve and first pair of gloves.

OR

a) Leave the first pair of gloves under the sleeve of the gown

b) Put a sterile sleeve of arms

c) Put the second pair of gloves over the sterile sleeve and first pair of gloves and enter the clean room

In short 1 pair under the gown sleeve and the second pair of gloves are over the sterile sleeve and first pair of gloves.

A1: I vote for the second one. I don't like the thought of pulling out the first pair of gloves to put them on top of the clean garments, when they were touching skin beforehand.

A2: Assuming you are using a sterile gown, then I would opt for the 2nd practice. During the 1st scenario, an operator's first gloves would become contaminated with skin bacteria while pulling the glove cuffs over the gown sleeve. This bacteria could then get transferred from the fingertips of the 1st gloves to the sterile sleeves and 2nd pair of gloves.

A3: In my opinion second option is more appropriate

A4: We use option 2 and spray the gloved hands with 70% IPA after each step.

EPPS Buffer in Microbiology?

I am designing liquid probiotics. I want to know about EPPS buffer. What is EPPS? (CAS No.16052-06-5). How can i prepare EPPS buffer? What is the use of EPPS buffer in Microbial growth?

pseudomonas spp on cosmetic products

I have a question regarding Pseudomonas species. While performing quality control testing on a finished cosmetic product we found some colonies growing on Cetrimide agar that was incubated at 30-35ºC, but I ´m still not sure if it is P.aeruginosa (no growth at 42ºC, oxidase positive, no strong green pigmentation on agar, and no strong fluorescent effect under UV light, although there is a greenish hue on the colonies). I would like to continue testing the product, but should I discard the batch if this is not P.aeruginosa? In the bibliography I found the main concern is P.aeruginosa, or Pseudomonas spp on pharmaceutical products, but I can find very little information about cosmetics AND Pseudomonas spp.

Background information: product´s challenge test complies with criteria A (Eur. Ph.), on retesting nothing was found. The product is a leave-on cleanser for the face, and I know some of our customers use it as a make up remover for the eye area. When incubating at 42ºC the temperature raised a bit (incubator issues) but not beyond 45ºC.

I am concerned about our customer´s safety. What would you do? Would you release this batch? I would like to know because, for more experienced microbiologists, this information may be enough to make a decision.

A1: I hope you are using more than cetrimide agar.
Please be concerned for any isolation of a Gram negative bacterium. Whether a pseudomonad an enteric, etc. these generally have potential to adapt and grow in the product, whatever its status rel. to a preservative test. Based on the information you shared, I'd not release this product and would be concerned for any product manufactured in the same time period between validated sanitizations.

Do complete identification and look hard at your manufacturing operations. You'll certainly want to eliminate contamination risk as soon as possible.

A2: Whether to keep the batch or not depends on what your micro specs are for your product. A lot of companies test for P.aeruginosa using the same methods which you have already performed yet the product specifications are usually absence of all gram negative bacilli. I would say get an identification such as Api 20NE from biomerieux and run your organism through that. They are cheap and good at identifying organisms in only 48hrs. If you have to have absence of all gram negatives to release your product then you should just scrap the batch but still do the ID because it would be beneficial for your investigation on the root cause of your contamination.

A3: Your concern is appropriate. There are many examples of pseudomonads causing problems in products for the eye.

Look at USP chapter <1111) (Pharm Eur 5.1.4) for guidance in evaluation of "other organisms". The concern is that the product should be safe, not that it should be absent of some index organisms.

There will be a webinar on this in a week or so - info at http://bit.ly/9XSBDQ if you want more information.

A4: Send the isolate for confirmatory genetic identification to a laboratory such as Accugenix. It appears as if you are performing selective screening for P. aeruginosa per finished product specifications as you had plated on to Cetrimide Agar, so if you get a confirmation, then yes you would have to discard the batch and

perform a full scale manufacturing investigation into the root cause (assuming you cannot rule out laboratory error in Phase 1 of the investigation). If it is not PA, it still does not mean the bug is not of considerable harm to the end patient. You will need to perform a risk-based assessment of impact to end user safety. I am coming from a pharmaceutical background, and do not know how much will apply to regulated cosmetics.

A5: Have you confirmed that the colony growing is Gram negative? We have seen Gram positive, spore formers growing on Cetrimide agar.

A6: I would only rely on definitive identification such as biochemical tests using identification kits or RNA to determine the presence of Pseudomonas aeruginosa as the test that you have already performed can not discard its presence.

(1)Typically Pseudomonas aeruginosa will produce the pigment but many external and nutritional factors could affect the microorganism's capability of producing pyocyanin and fluorescein. In fact pigment production could be affected by the type of peptone used in the base. I wouldn't limit my decision on this characteristic as not necessarily your isolated strain will exhibit it strongly.
(2)Pseudomonas aeruginosa can also lose its fluorescence under UV if the cultures are left at room temperature for a short time. This could be the reason why it was not observed strongly.

Although not specified, it seems to me that the growth was observed in enrichment. Did you have any recoveries in total plate count? If no detections were observed in TPC and the cetrimide growth was weak, I would have expected no detection in retests as it seemed to be weak and in low numbers. I have you tested additional samples?

In my personal opinion, if the presence of Pseudomonas aeruginosa is confirmed via an identification kit, I would not release the product as this bug is an opportunistic, drug resistant pathogen and spoilage organism. Your product specifications should be your guidance on what is to be considered objectionable.

A7: To clarify, my comment was meant to address the scientific relevance of using in-house isolates vs. type culture organisms. As we all too often experience, regulatory relevance may not be one-in-the-same.

On February 2nd of this year, you replied to a member's question regarding GP of water test media...

"First we should remember that the purpose of growth promotion testing is to confirm that the R2A medium has been correctly prepared and shows lot-to-lot consistency.

For this QC function we can select cultures appropriate for the medium as recommended by the AWWA/APHA standard methods, the compendia and the media manufacturers.

The selection should consider the type of bacteria that may be found in your water system as well as the incubation conditions.

Given that most companies are using the Ph Eur requirement of 5 days at 30-35 degree C B.subtilis (spizizenii) ATCC 6633 and P.aeruginosa ATCC 9027 plus an environmental isolate like R. picketti may be a good choice."

Since you referenced the use of an environmental isolate on February 2nd, how would you reconcile this with the information you presented in your April 30th posting? No insult intended. I'm just curious where you and other industry leaders stand on the use of in-house isolates.

A8: I agree with [name redacted], I would not have any gram negative organsim in the product on the plate count or upon enrichment.

A9: Pseudomonas spp. can form biofilms that protect them from detergents and sanitizing agents. This makes them extremely hard to get rid of in a manufacturing area.

Pseudomonas spp. can be multi-drug resistant. Bacteria that form biofilms are more difficult to treat with antimicrobials. Antimicrobials may fail to penetrate into the biofilm. Bacteria within biofilms can have increased (up to 1000-fold higher) resistance to antimicrobial compounds. Bacteria can also express new and sometimes more virulent phenotypes when growing within a biofilm.
You may want to read the article: Pseudomonas-The Nemesis of the Cosmetic Industry
By: Donald Orth, PhD
http://www.cosmeticsandtoiletries.com/research/methodsprocesses/61216642.html?page=1

A10: May be this organism is Burkholderia cepacia which resembles pseudomonas in many ways (Recent taxonomic changes have resulted in the genus Pseudomonas being split into multiple genera including Burkholderia, Ralstonia, and Stenotrophomonas). Please check your preservative system and efficacy against pseudomnas sps. The main source of B.cepacia contamination is through water and soil.

It is obvious that contaminated product should not be released in to the market.

A11: [name redacted] makes a very good point. Exclusive reliance on so-called selective media is a mistake. Cetrimide, PIA, EMB, MacConkey - each of these can recover non-target bacteria and even fail to recover target microbial isolates. These were largely developed to recover qualitatively clinically relevant target bacteria from mixed inocula.

A12: I'm in agreement that using biochemical reactions of organisms from just selective/differential microbial growth agars as a final identification call of an isolate is a mistake. I feel that these color reactions on these types of agars is just a presumptive identification. I would be very hesitant to make a final identification call of an isolate just based upon an isolates biochemical reactions from selective/ differential microbial growth agars that are commonly used in microbial testing. As a microbiologist, I think that it is important to put a presumptive identification into its proper perspective. It might be or might not be the organism that you are looking for by using these agars. I think that the final identification call of an isolate from selective/differential microbial growth agars should be based upon using the technological advances(e.g. genotypic or phenotypic biochemical reaction databases) that have become readily available in the last 20 plus years for determining the proper identification of bacterial isolates. Without doing this, how do you make a decision of whether an organism is objectionable or not without having a proper identification to the species level as the first step in your decision process. I would be very hesitant to start making my decision on the acceptability of an organism in a finished product just based upon a presumptive identification. In reading several postings on this subject, I do agree that the presence of Gram-negative bacteria in any type of finished product is objectionable no matter what is its identification.

A13: Hi all,

It intrigues me that the compendial test for Ps. aeruginosa uses an agar (Cetrimide) that will, to some degree at least, prevent growth of some of the objectionable species for which Ps.a. indicates (biofilmers , when it comes to water systems, and outright pathogens among them when we test topicals). Since we now know we are searching for a broad group, or numerous genus/species within, and not just Ps.a (for want of a better term: pseudomonads, or Ox positive GNRs?) we should ask the question How are we to screen for objectionables from a cetrimide plate? Our only alternate is the parallel R2A plates? I think not!

At least we have a fair chance of picking up "other" objectionable enterics when using e.g. XLD, MAC or VRBG media...

Surely now we are all talking about "Objectionables", we should get rid of Cetrimide agar and try e.g. PIA?

A14: ApI identification would work for Id of organism. even if it is not Pseudomonas, any gram negative most of the time multiplies and reaches out of spec count. which can detoriate product/ injurious to the user. We reject the product with any gram negatives, pathogens also any other organism which exceeds TPC spec.

A15: Good point and I think the answer is not using more of these problematic media but for all of us to "be" microbiologists - use nonselective media (whether or not selective as well) and identify growth. A

highly trained microbiologist isn't needed if all that counts are correct method execution and observation of growth and color changes. But we know the error of presuming all objectionables of environmental origin react precisely, for qualitative recovery, as the clinical bugs used to development and qualify these selective media.

A16: The selective media have its purpose when one is selecting for the particular organism from a mixed culture. As in Clinical specimen where you have the normal flora along with the pathogens or in Herbal products where there is a variety of organisms and you need to enhance the growth of specified organisms. But in the cosmetic or drug products where we are looking for organisms, an enrichment followed by isolation on a general purpose(non selective isolation) and then ID from there, helps. considering the growth limitations of different selective media, use of non- selective media along with any of selective gives better direction to a microbiologist.

FDA registration question

Has anyone heard of a company called Registrar Corp? They claim to assist businesses with FDA compliance. My company would basically pay them $995 a year to help with the FDA registration process and act as the "registrant contact" for our company.

My company has decided to become FDA registered and I am wondering if it is worth having someone else do the filing and upkeep for us since we are a small company (20 people), or if someone can point me in the right direction on how to go about submitting an electronic form.

A1: Are you registering with CDER or CBER? If CDER, it is an on-line process are fairly simple. If is CBER, is still paper based on more time intensive.

A2: Hi I did min myself in about 10 mins. I looked into that company it seemed like a scam. Better luck using Scott Sutton or Dave Porter from vectech.

A3: I would be careful of this - the process is not really all that complicated.

Can someone who has recent experience provide the web site and process for this?

neutralize antimicrobial activity

If you have a non-sterile pharmaceutical product that has antimicrobial activity that has to be neutralized according to the harmonisation of the pharmacopeias, do you add the neutralizers (Tween 80 and Lecithin) to the diluent or to the Tryptic Soy Agar while trying out the suitability of TAMC/TYMC?

A1: Neutralization schemes often include a combination of neutralizer in either the liquid medium as well as they plating media, or both. Before you begin executing your Suitability Testing, "play around" a little and see what works best. Be sure to demonstrate low neutralizer toxicity as this is often overlooked when people only show high/sufficient neutralizer efficacy. When using multiple neutralizers and membrane filtration, you want to ensure the media employed and the action of the filtration are not inherently adversary to the microorganisms which would lead to false positives are artificially low results.

A2: You can do however you like, usually beginning with the diluent and if it doesn't work try it to the TSA. The important thing is that the suitability test works. It's important to allow the neutralizers a time in contact with the product before adding the microorganisms.

A3: It is my recommendation that you add Tween 80 and Lecithin to the microbial count diluent. I believe it is stated in the USP that neutralizing agents are added to the diluent. If you add Tween 80 and Lecithin to Tryptic Soy Agar, you are using your own version of Microbial Content Test Agar which is commercially available. I believe that Microbial Content Test Agar is not listed in the pharmacopoeias as an acceptable microbial growth agar.

A4: USP guideline states to add neutralizing agent to diluent or plating media or to both.

A5: The problem is that I have several finished products and raw products (even pharmacopeial raw products like paracetamol and zinc gluconate) for which adding Tween 80 (5g) and Lecithin (0.7g) to the diluent doesn't suffice to approve the suitability results. I don't know what to do?

A6: With Zinc gluconate, the problem with your neutralization issues is with the Zinc portion of the compound. I always found products containing Zinc are difficult to neutralize because Zinc has antimicrobial activity. It is my understanding that Paracetamol or Acetaminophen is also very difficult to neutralize the antimicrobial activity. I believe that Acetaminophen is a aniline derivative from what I remember of Organic Chemistry. These types of compounds have been found to have antimicrobial activity. Besides using Tween 80 and Lecithin as chemical neutralizers, you might want to try the addition of histidine or other chemical neutralizers to the Tween 80 and Lecithin microbial count diluent. With the Zinc gluconate, I wonder if the addition of a chelating agent such as Disodium EDTA would work in preventing Zinc from having an effect, but I have never tested this hypothesis. I always found dilution in combination with additional chemical neutralizers to the Tween and Lecithin would work. In addition, you might try dilution in conjunction with the chemical neutralizers. Instead of a 1:10 dilution, perform a 1:100 dilution of the sample in the microbial count diluent. Furthermore, I found a trick by increasing the Petri plate size from 100 x 15 millimeters to 150 x 15 millimeters and use the additional microbial growth agar for plating in these Petri dishes to dilute the sample diluted aliquot further. In the end, you might not be able to neutralize the antimicrobial activity of these compounds against all of the test organisms that you are using to validate your test method. I believe that there is a statement in the USP microbial limits testing section dealing with this type of situation if you are not able to completely neutralize the antimicrobial activity against all of the test microorganisms, but I do not remember the actual wording.

A7: Add more tween 80 , try until 5%

A8: Options can be
Increase the test dilution compatible to your spec.
Increase the concentration of Tween and/or Lecithin
Incorporate the neutralizers into diluents and plating medium.
Use a larger diameter petri dish (may be higher price) for plate counts that will hold more agar and helps out to dilute the inhibitors.

A9: I normally use diluent like Modified Letheen Broth or D/E for all products and verify if the spike organisms can grow. You may find some examples of neutralizers of antimicrobial activity of several preservatives in Annex of ISO 22717, 22718, 21149 etc. or other related standards. In addition, list of validation techniques may be found in: Sol Singer. 1987. The use of preservative neutralizers in diluents and plating media. Cosmetics & Tiletries. Vol. 102.

A10: Another question for routine testing: Where do you get your diluents with neutralizers? Because I add them individually every time, but for routine testing of products that will be too time-consuming.

A11: Neutralizer should be added during microbial limit test when your product contains antimicrobial ingredients even may be preservative which are used in oral liquid preparation. Tween 80 or Lacithin are major neutralizer which are used for Oral liquid preparation and few antiseptic test. I was in a company where there is a product with combination of Chlorhexidine gluconate and Cetrimide solution in topical preparation, for total aerobic count of that product I inoculated Lacithin and Tween 80 as neutralizer and validated that product. I got excellent result and performed routine test at several years.

Media challenge with environmental isolates

My question is about requirements for challenging EM and/or sterility test media using environmental isolates recovered from the manufacturing facility. We currently perform it quarterly on one lot of each type of EM and sterility test media, but I have heard mention of it being performed on each lot. What is the

industry standard for this type of testing? Is there a specific regulatory requirement, observation, or other rationale for a specific frequency? Is there any differentiation between EM and sterility test media? Thanks in advance for your help.

A1: We are a raw material producer, inspected by the FDA, we have been advised to add an environmental isolate to our GPQ tests, but we only do an annual test on the EM media.

A2: There is no official USP test for water monitoring. In general, I do not support the wholesale use of in-house cultures for growth-promotion testing.

A3: While I fully agree with you that an environmental isolate, once cultured for several passes in the lab is no longer the same bug, one of the very first questions we are asked about our environmental program by almost every regulatory inspector is if we growth-promote with environmental isolates. Another case of compliance vs science.

A4: Whether the media is used for EM or for sterility testing it should undergo GPT test. On sterility test as far as Positive control is concerned you can put GPT on every sterilization lot of the media prepared. And for EM every newly opened dehydrated media container should be tested for GPT with the environmental isolate. For environmental isolates you should maintain the matrix and analyze it on quarterly or half-yearly basis for any new isolates. If any new isolate is discovered that should be properly documented identified and should be included in the GPT of the EM. The basic idea behind using the E isolates in GPT is to check that the media is potent enough to support the growth of isolates in line with standard cultures.

Eyewash stations

I'm curious to know which acceptance criteria have you established for microbiological test of water from eyewash stations.

A1: Given the intended use of the eyewash - i.e. on potentially injured eyes - I would have thought that the fluid needs to be either sterile or of extremely low microbial count.

A2: I agree with you. The problem is that eyewashes are not bottles with isotonic sterile solutions. They are stations eyewashes fed with potable water. Maintenance procedure is a monthly disinfection and a weekly flushing during 3 minutes. This is not sufficient. Counts are acceptable only the first or the second day after disinfection.
Which maintenance/disinfection method and acceptance criteria do you recommend?
Also I would like to know experiences of others PMF members.

A3: From my experience all eyewash systems were never tested for microbial counts since the system utilized municipal water. That being said the system was flushed for three minutes each month and the flow rate was checked and in working order. I have seen some facilities obtain portable eyewash stations or kits in the event of a mishap (but these would not satisfy the recommended usage of lushing the eyes for at least fifteen minutes).

A4: Let me insist with my question. Does anybody test the water from eye stations?. What is your experience?

A5: Are you referring to free standing stations, or are you referring to potable water supply stations that are near sinks. For many free standing stations, the water supply comes with an expiry date, but few abide by the information provided as the Environmental Services group typically handles such stations and not quality.

A6: If one were going to be sensible, one would monitor post-flushing daily and determine how often one HAD to flush to maintain acceptable water quality (i.e., AT LEAST potable quality, preferably far less)- but I've honestly never been anywhere where that was done. Most places finally gave up and got disposable sterile eyewash squeeze-bottles and disconnected the tap-water ones as being un-maintainable.

A7: I´m referring to water supply stations. According to safety engineers the portable bottles are not sufficient to wash efficiently the eyes. A self-audit ask for the testing of the water from the stations.

A8: After almost 30 years of working in various types of laboratories with eyewash stations, I have never seen one tested. With nearly 20 years of experience in pharmaceutical microbiology, I have never been part of testing eyewash stations or had it come up in an audit or inspection.

A9: If, as [name redacted] infers, you have such a diligent OHS dept. that they ask for water quality data from the micro dept. then that's very good news for Safety at Work; although as most respondents also note, even good potable quality is clearly not safe for eye washing according to regulatory standards for products intended for application to the eye. However, as an accredited Safety Officer, I can tell you that a potential eye infection acquired by flushing poor quality potable water into de facto damaged eyes, is a far lower risk than just trying to pour a few milliliters of sterile saline into eyes that have been splashed with something really nasty! MSDS documentation and OHS will always trump any Quality issues, especially when it comes to something as evocative as eyes. They usually suggest flushing for 15 minutes, and then see a doctor (who will then assess the need for antibiotics). By all means, make the eyewash station as clean as possible by regular flushing, but don't bother trying to attain any sort of consistent quality; not possible in a dead-legged potable supply. This equipment is used for serious emergencies and all micro issues go right out the window in order to save someone's sight.

A10: No. Testing from the eyewash stations has never been done for microbial count from my experience (20 years)...I believe the immediate concern is to flush the foreign object (chemical) for example first (rather than be concerned with the "normal flora" of potable water. Maybe this is antiquated since it is based on what a person would do if they were "at home" and it seems that your company is going the extra mile.

A11: This is a study in the literature.

See Examination of microbial contaminants of emergency showers and eyewash stations

J Indust. Microbiol. Biotechnol. 20 (3-4) 129-143 1998

A12: We have tested water from eye wash stations in the past to try and see how long they should be left between flushing and how long they could be flushed for before use - obviously this has to be quite short in an emergency. We also did a similar exercise on our laboratory drench showers. It's not regulatory, wouldn't come up in an audit (maybe a HSE audit?) but is a good thing to do for safety reasons.

We tested the water every day for periods of time ranging from two to four weeks and from the data initiated a regime that the showers and eye wash stations are flushed for a certain amount of time every month and when used in an emergency are flushed before use. The time for this has to be weighed up against what the emergency is and how much time you have.

Are the station fed from mains water? The wash bottles are handy but sometimes you have to wash the affected area for a least 10 minutes.

La Calhene Isolator for Sterility testing (ISOTEST)

Could anyone share his/her experience with me concerning the "Getinge La Calhene" rigid wall isolator for sterility testing?

We recently acquire the equipment in our Lab, the validation went on fine in spite of some minor issues here and there.

But since we started routine use about a month ago, we've been having problems with the hydrogen peroxide injection system.

Is anyone using this system for sterility testing?

A1: We have such an isolator since 12-2008 and do not have any problems since. Be sure to use the right hydrogen peroxide quality. And from time to time you need to clean the injection part when necessary (ones every half year should be sufficient).

Furthermore we can say that it is working fine and we do not have any problems. We use the system (decontamination) at least 4 times a day and 5 days per week.

I do not know how many times per week you decontaminate the isolator? It could be a problem that the system is not running well if you use it for instance ones a week.

A2: I have used La Calhene isolators with Steris VHP generators awhile back. They are very well manufactured isolators, I had occasional electrical issues related to automatic values etc, but nothing major. When you say you have problems with hydrogen peroxide injection system, what exactly do you mean. Do you get an error that the system cannot inject peroxide?. Do you have an integrated system, isolator built in with hydrogen peroxide generator? Hydrogen peroxide generator have injection values, they might need to be cleaned, manufacturer's recommend you use their brand hydrogen peroxide to avoid clogging the values.

It sounds more like the issue with the VHP generator than the isolator itself.

A3: The equipment has an integrated biodecontamination system; it's relatively new, just a couple of months old.

I am in contact with the manufacturer and they will be coming around to have a look and try to fix the problem, they are suggesting mechanical issues with the hydrogen peroxide pump or/and the detection system.

They don't seem to have a lot of history with the integrated system.

microbial limit test method for antibiotic ointment

Does anyone have a reference to a test method for microbial limit testing or presence or absence testing for non sterile over the counter antibiotic ointments such as bacitracin or polymixin. - Use of isopropyl myristate to solubilize ointment? - if membrane filtration is used, what type of filter used? - any appropriate neutralizing agents?

A1: We had a non sterile topical antibiotic ointment in my previous company. The product was paraffin - based and contained 3 antibiotics - neomycin, polymyxin and bacitracin.

The microbial limit testing method comprised dissolving 1 g product in 100 ml sterile isopropyl myristate (maintained at about 42 - 44°C in an incubator, filtering the solution (in duplicate) through a sterile 0.45 micron cellulose nitrate membrane filter, washing the filter with 5 x 100 ml peptone water (Pre-warmed to 42 - 44°C) containing 1 % tween 80, transferring one filter to a TSA plate and the second filter to 100 ml TS broth and incubating at 30 - 35°C for 5 days for total viable count and 48 hours for detection of pathogens.

You need to ensure that there is no entrapped moisture in the isopropyl myristate prior to addition of the sample. Also ensure that the solution of product has been completely filtered before washing the filter paper with the peptone water.

We had also observed that if we happened to add more than about 1.3 g of the product to the isopropyl myristate, the solution would become very viscous and difficult to filter.

Please validate the quantity of product and number of washes.

A2: I did not find any reference for Microbial count of Bacitracin Zinc and Polymixin B ointment. If you want to carry microbial count of any ointment, isopropyl myristate may be used or heat at 45 degree celc. before pouring into plate. Pour plate method may be used for microbial count of ointment. Reference for test of Ointment have specification in BP, chapter- Pharmaceutical Microbiology Quality Control, category- Topical Preparations as below :
Total aerobic count not more than 100 cfu per gm for bacteria and fungi. Ps. aeruginosa and Staph. aureus must be nil.

Water System Routine Monitoring Frequency

What is the sampling frequency for microbiological and chemical monitoring of user points and water storage tanks after the completion of water validation? If there is 10 WFI user points and 15 Purified water points, how to spilt the sampling points.

A1: There is no one simple answer or standard. Your validation, use, maintenance schedules, etc all will determine the frequency and manner in which you perform routine monitoring of the system, as it is intended to determine shifts in operations and quality output. It is expected that the routine monitoring program is scientifically and risk based.

A2: During validation of water points, you had to sanitize the water system, so you got good information that , for example, when you sanitized the system once a week you got acceptable results, so it is enough once a week to sample your points with routine sanitization. This is the minimum acceptable practice.
For WFI, it should be sampled with each preparation to be in the safe side & remove any probability of Suspicion or doubt.
Our practice:
For purified water, twice a week.
For WFI, every preparation day or at least twice a week if there is no work during this week.

A3: For purified water all user points should be considered atleast once in a week and for WFI you have to sample on daily basis.

acceptance criteria for growth promotion

Anyone knows if you inoculate with <100CFU and you obtain 1-2 CFU, would that still be acceptable? Anyone use a range 10-100CFU as an acceptance criteria? I cannot find any regulation on that. USP <61> just state <=100CFU.

A1: We use 10-100 CFU/ml for ATCC cultures but *<* 100CFU/ml for House Isolate cultures when carrying out growth promotion testing.

A2: The accep. crietria shall be factor of 2. Ex. If u inoculate 40 cfu accep. crietria shall be between 20 and 80 cfu.

A3: Definitely It is not acceptable because bacterial recovery should be at least 50 percent during validation when 10 to 100 cfu are added. If you add into broth media that is different matter. In that case growth must be obtained.

A4: Have anyone done growth promotion and obtain results below 50 CFU (if inoculate with <100)? would that be still acceptable since it is less than the factor of 2 (50-200)?

A5: As USP, the min. acceptance criteria is 70% of the original inoculum, while not mentioned the up limit.

A6: Exactly [name redacted] is right. Acceptance Criteria should be + / - 2 fold of inoculated microorganism.

A7: It is not very difficult to obtain the growth promotion result below 50 cfu. If you deactivate any antimicrobial properties properly from your product you can find these types of result. Now there are many companies which produce 10 to 100 cfu validation kits such as Microbiologics. In last 10 years I used these kits and that result was very nice. Can anybody share with this issue ?

A8: I also use micro-organisms from Microbiologics, but sometimes I also get low values. I always plate from the same micro-organism suspension on TSA to compare and that is my +/- 2 fold standard. Our supplier told us that the concentration mentioned on the box is only an indication and no guarantee of the specific concentration (also the number decreases during time), so we always test against a TSA control.

A9: If you don't get 50% recovery, is that mean the media fail growth promotion? not all organism can give a 50% recovery for growth promotion.
How you do compare your + / - 2 fold of inoculated microorganism.? Do you just assume your re-constitute organism is 100?. What if it' s really only give you 30 CFu when you pipette 0.1ml.? how would you compare to your actual media since it is not 100 anymore?

A10: You're right, you can't assume your inoculum is 100. Quanticults and other available standardised micro suspensions contain 10 -100 cfu/0.1 ml. You have to have count plates on another batch of media so you can compare it to the new batch and then work out your recovery.

Proposed revisions to the ISO 14644 Total Particle Counts

Has anyone seen the proposed revisions to the ISO 14644 Total Particle Count document.
One revision they are proposing is to not use the square root of the room area (in square meters) to determine the number of particle sites per room. The 95% UCL will be changed too. The change in the 95% UCL will require that more particle sites be sampled.

When I heard they were revising ISO 14644 I thought they were going to remove the 95% UCL, but did not know they were going to replace it with something more extensive. The reasoning is the finally admitted that the 95% UCL only works if the room is static. Under dynamic conditions workers make the underlying assumption for the 95% UCL void.

Does anyone have any information on the new revision?
I was surprised to hear the EU is going to go along with what ISO is proposing. Presently the EU does not do the 95% UCL.
It sounded like they wanted to increase the number of sites where sampling of 1,000L (Cubic meter) would be required. The EU has started saying this too.

A1: Despite the fact that the author is hung up on settle plates, here is the skivvy:
http://www.cemag.us/article/changes-affecting-eu-gmp-and-fdas-cgmp-non-viable-particle-cleanliness-cleanrooms

A2: The draft will no longer have the 95 % UCL for 2 to 9 location test sites. Also the draft will have a different method of determining the number of test site locations which will be based on providing 95 % confidence that at least 90 % of all locations ≤ the limit.

The draft still needs to go out for translation before it is ready for DIS vote. The translation will take several months and the DIS vote is 5 months. The proposed changes with the 95% UCL may not make it into the final version of the standard so there is probably no reason to get too excited about this yet. For more information you can contact IEST (www.iest.org), which serves as the Secretariat of ISO/TC 209.

Garments cleaning requirement for Pharmaceutical manufacturing facility

I wish to know about the cleaning/washing facility requirements like environmental conditions of area where clothes are washed, dried and stores for Both STERILE & NON STERILE PRODUCT MANUFACTURING.Also some testing requirements & details about garment quality/life. If any reference standard soft copy is shared will be highly appreciable.

A1: One reference would be the IEST RP3.3 document. It covers a lot of what you ask for cleanliness characteristics and testing but does not define sterility requirements. They would be specific to pharma/biotech/MD/Hospital. And they are simple, 10eu3 - 10eu6 depending on the material's ability to be gamma sterilized that is based on the bioburden of the final gown product. The document can be and can be obtained at http://www.iest.org

A2: The following references may help for the nonsterile garments.

ISO 14698-1 : 2003 Cleanrooms and associated controlled environments - Biocontamination Control Part 1: general principles and methods. Annex D: Guidance on determining the bioburden of textiles and Annex E: Guidance on validating Laundering Processes

EN-13569 : 2001 Cabinet roller towels and performance requirements and processing EN 14065 : 2002 Textiles - Laundry processed textiles - Biocontamination control (this is risk analysis standard like HACCP)

There are two German standards for Bioburden of linen both wet and dry, water and surfaces RAL-GZ 992/2 and RAL-GZ 992/3.

There are a number of suppliers including EMPA test materials , BAG Health Care GmbH, that supply test materials that are treated with protein, microorganisms, blood etc that can be used in the evaluation of the washing process.

The hospital laundry standards may a useful area to start your search.

Sample Quantity for MLT Validation?

I want to Know minimum Qty. for MLT Validation?
Can I apply dilution scheme further from 1:10,1:50,1:100 dilution?
How will we cope up with Product Dilution and Culture dilution during dilution scheme?

A1: Minimum Qty of MLT validation is 10g , unless otherwise mentioned in monograph.
You can apply dilution, if recovery is not satisfactory. In this case, increase the plating no. to cope up specification. (for 1:100 dilution do 10 duplicate or triplicates)
For culture dilution, prepare 1000 or 10000 CFU/0.1ml (for 1:10 dilution use 1000 cfu/0.1ml and for 1:100 dilution use 10000 cfu/0.1ml) to get 100 CFU.

Rapid micro methods for sterility testing

Alcon Labs has FDA approval for using a solid phase cytometry rapid method for finished product sterility testing. Vendors of other rapid methods that have developed ATP bioluminescent and CO2-detection systems are also working with their customers in validating rapid sterility tests. Is anyone on the list evaluating or validating any of these or other rapid methods for sterility testing?

Test for specified microorganisms

USP<62> describes tests for the "absence of seven" different specified organisms.

With how many of all seven organisms should we perform test for specified microorganisms if there is a vaginal product and not covered by monograph in the USP ?

Do the tests depend on non sterile dosage forms (in these case: oral use, nasal use, vaginal use..)?

How many people test their product for all bacteria and C. albicans described in the USP chapter" 62 Microbiological examination of non sterile products: test for specified microorganisms?"

A1: The microbial test requirements and limits for the products for vaginal use should be TAMC (100 cfu/g or mL), TYMC (10 cfu/g or mL), P.aeruginosa (should be absent/g or mL), S.aureus (should be absent /g or mL) and C.albicans (should be absent /g or mL).

For the same information refer informational chapter in current USP <1111>.

A2: The recommended microbiological quality requirements for each dosage form are found in USP <1111>.

A3: You are misinterpreting the compendial requirements. The seven organisms are examples of what may be objectionable in your product. There are probably many different organisms that could be found objectionable should they be in your product. The indicator organism concept is now gone from the compendia and replaced by the requirement that objectionable organism be absent. It is up to you to determine what is objectionable in your product and to defend that position. The compendia (USP <1111> has guidance on how to determine if an organism is objectionable. There is no "bad bug" list for pharmaceuticals as there is for foods.

A4: Since you are referring to the USP chapters, I am assuming that you are concerned with testing for the US although my comments apply equally to the EU. Since you do not have a monograph you are not restricted to any of the organisms. What should concern you is absence of objectionable microorganisms. These organisms are expected to be defined by you, the manufacturer. I suggest you look at USP <1111> for a start. Again, the expectation is absence of objectionable microorganisms.

Quantitative test for Bile-Tolerant Gram-Negative bacteria

Can anyone share their experience with validation of the quantitative test for Bile-Tolerant Gram-Negative bacteria? We observe a significant variation of the results when SCDB inoculated with <100 cfu of E. coli / P. aeruginosa is tested per the procedure outlined in USP <62>. Our customer set acceptance criteria for BTGN bacteria at NMT 100 cfu/g.

Enumeration test at end of stability

I am working with a Rx shampoo and currently perform TAMC , TYMC and absence testing for release. AET is performed for samples that have been put on stability. My question is should release testing be performed in conjunction with AET at the end of expiry for stability samples, or is our current practice of AET only on stability samples suffice. Any help would be appreciated.

Antibiotics - method suitability studies

I am interested in your views concerning antibiotics and their "microbiological purity".

It seems to me that a lot of people are trying to neutralize their antimicrobial activity, in order to obtain recoveries of inoculated test microorganisms. That costs a lot (especially neutralisers, for example

lecithin), and it can be very frustrating to search for a neutraliser where there isn't any (when the antibiotic is so strong that it yealds 0% recovery even when 1:10 000 is plated!).

Pharmacopoeias say that we should try to use neutralisers, but when we fail to obtain the microorganisms, we can say that failure of the suitability testing is attributable to the nature of the product and that the product is not likely to be contaminated with types of microorganism used in the study.

So, what do you do?
Do you spend a great deal of time and money in order to find the right neutraliser, or do you stop at the very begining and use the above mentioned pharmacopoeial explanation?

I am facing a strong antibiotic at the moment, azithromycine - all test bacteria failed to grow in the presence of the product even when 1:1000 sample dilution was plated. I didn't find any specific neutralisers in the literature, and I already tried general neutralisers like lecithin and tween. Nothing worked. And filtration, of course, is out of the questions, since the product is very thick and leaves a lot of small particles in the solution. What would you do?
If I could go back in time, I probably wouldn't go into the whole neutraliser search - it cost a lot, and it gave me no results.

A1; I hope you did all this under cGMP rather than just "experimental toying around". The reason I say that is because I think if you show multiple attempts using an array of neutralizers, dilutions, etc that are suitable for the material under test, and this is done under strict cGMP guidelines with pre-approved protocol or following an SOP, then you can write up a nice justification in your final summary report explaining the scientific rationale as to why you think the material isn't a risk. I think often its how you come to a conclusion, and how wells its documented.

A2: I am also facing strong macrolide antibiotic, and trying to neutralize the antimicrobial activity using divalent cations to run sterility test (microbial limit test to follow). Like to see if anyone successfully used cations to neutralize the antimicrobial activity.
http://jac.oxfordjournals.org/cgi/content/abstract/17/4/481
http://jac.oxfordjournals.org/cgi/content/abstract/18/2/185
http://www.faqs.org/patents/app/20100075368

In general I take following steps when faced with difficult to neutralize antimicrobial activity (for microbial limit tests): check possibility of using filtration method, if not proceed with highest dilution>>>Google for suitable neutralizers (neutralizers discussed in USP are preferred)>>>highest dilution+neutralizers (couple of trials)>>>bang my head in to anything that is not moving (brick wall is preferred)>>>Google>>>3 to 5 trials of neutralization using different neutralizers or combination>>>bang my head again>>>calm down>>>write a report saying that sample is difficult to neutralize, and use method that resulted in maximum neutralization for regular testing.

I guess some of the steps are not necessary! Most of the time I am lucky to find neutralizers before I reach "calm down" step (which is most difficult part of the neutralization scheme).

Forgive me, I think there are better methods to neutralize the antibiotics.

A3: Especially for the antibiotics we can implement the usage of neutralizers such as

a) Betalactamase - supplied by Oxoid
b) Betalactamase (Broad spectrum) - supplied by Oxoid
c) Penicillinase - BD difco
d) Combination of penicillinase and Betalactamase - both the above.
e) Metallobetalactamases etc.

According to the BUSH Betalactamases are classified into Four categories depending on their substrates.

a) Cephalosporinase (Amp C) - Class-I
b) Penicillinase (ESBL) - Class-II
c) Metalloenzymes - Class-III
d) Most resistant Betalactamases- Class-IV.

Study :

First we have to calculate the concentration of antibiotic in the dilution, followed by use this enzyme concentration at different levels to know where the complete neutralization is achieved and also prove the same using HPLC data so that it will be a supporting data for you explain to the auditor.

Note : Pour the media into the respective plates at 40 - 45 C as these enzymes are very sensitive to the temperature.

Pipetting of inoculum or solutions

I have a few questions which i think you can help me out in solving.

1. Can we to take volume of inoculum/solution for adjusting or making up to certain volume in these figures like 1.25, 1.5, 1.75 etc or there is any rule to follow while pipetting ?

2. Why should we incubate SCDM agar/broth first at 25°C and later at higher temperature in Environmental monitoring and Media fill test?

3. For Class 1000000 area we kept Bacteria limit as 50 and fungal limit as 25 per plate. In this case if plates are first incubated at 25°C , there are chances that even 5-6 colonies of mold may cover entire surface of media plate and there may be no chance for bacteria to grow if we incubate later at 30-35°C for bacteria. can you please clarify?

4. Do we need to carry out preparatory test for microbial limit 3-5 times to validate the suitability of the test method.?

Growth promotion test for media

Please clarify.

Do we need to perform Growth promotion test for receipt of dehydrated culture media lot/batch OR for a sterilized lot /batch of prepared media.? Please give the reference standards in this regards.

2. Can we manage with the positive controls at the time of analysis?

A1:
1. The answer to your question can be found in the USP-NF General Chapter <1117> "Microbiological Best Laboratory Practices" under the "Quality Control Section".

2. No, positive controls are not a substitute of your growth promotion tests. It is critical that you challenge your media with low levels <100 cfu. Please refer to USP <1117> and USP <62>

A2: In regard to your first question, please look at <61> in the current USP:
Test each batch of ready-prepared medium and each batch of medium prepared either from dehydrated medium or from the ingredients described.
Chapter <71> (Sterility Tests) of the current USP:
Test each lot of ready-prepared medium and each batch of medium prepared either from dehydrated medium or from ingredients. 6 Suitable strains of microorganisms are indicated in

And Chapter <1117> (Microbiological Best Laboratory Practices)

In regard to your second question, some years ago I took on the responsibility for a microbiology lab and soon discovered that this was exactly the approach that they had taken in regard to growth promotion. Of course, the universe being what it is, the FDA showed up soon after I made this discovery. I tried to get them to buy the use of positive controls in place of growth promotion, but to say the least, they were not interested in buying that story.

A3: AS per USP GPT shall be performed each batch of medium prepared fro dehydrated medium or from the ingredients.

Positive control is reference for your test procedure.

A4:
1- We don't perform any test on receipt for dehydrated media. You must do it in every batch of prepared media, that means you should perform the test every time that you prepare a batch (sterilized batch).

2-No, you should have a separated area for the positiv controls. In fact, several audits here have asked about it. We have a biosafe hood to perform this tests.

Amphotericin interactions?

Hi all. Recently my company changed supplier of Amphotericin B. The new API has Amphotericin A and X in it as well as B (at less than 5%), while the old supplier gave us product with either no, or very small amounts of Ampho A in it.

An examination of the changes and distinctions between product we can assay successfully and product we cannot has identified the change in API as the most likely cause our assay falling over (10-20% less activity than there should be).

As the only apparent difference between each supplier's product is the level of impurities, I am wondering if anyone can answer the following questions (failing that, anyone know of a good text discussing Ampho B, A and X?):

Does Ampho A inhibit the action of Ampho B?
Does Ampho A diffuse better through agar than Ampho B?
Does Ampho A affect the stability of Ampho B?
Is it possible to deactivate Ampho A and X without affecting Ampho B?
A 30 year history of Ampho B use at my company indicates it is quite stable at room temp, both in liquid and tablet presentations. Do others have a different story?
When looking at zones of inhibition, what would cause a halo effect? (diffusion out and up of wells? not enough culture in the seed agar? not enough liquid in the inoculum (20 ul in an 8mm well that is about 3-4mm deep), other?)

My own hypothesis is the new API is bad and that is giving us issues. The non microbiologists who run the show are convinced our assay has spontaneously ceased to work (despite all our other antibiotic assay working just fine). If I could prove either I'd be a happy little vegemite.

Any insight into this issue would be appreciated.

A1: Let me guess, some genius though he could save money by switching suppliers.
You may consider another point, concentration. If you added X grams of the old stuff (100% amphotericin B) to the formulation, and you are adding same amount of the new stuff, then you are adding less amphotericin B. Those contaminants make up a percentage of your input.

You can correct this two different ways. One, increase the amount of the new stuff so that you are delivering the same amount of amphotericin B as before. Two, buy the right stuff.

mad scientist costume

Here is a bit of fun - I am having a fancy dress birthday party in a couple of months and I want to dress up as a "mad" scientist. Was wondering if any of you lovely people out there had any cool suggestions for my costume? :)

A1: I think that you need a good hair do. Use spay and gel to have it sticking up crazy. You may even want to "rat" or tease it too this will help it hold up through the party. I hope you have a good time!

A2: Samantha and Julie have me thinking of the Spawn of Frankenstein thread of several months ago. A brilliant short break from the usual...

Craft the Groucho Marx glasses, nose, and mustache get-up but with some wonderfully geeky safety glasses outsized for your face.

Have fun!

A3: After you tease your hair and make it stand straight up, throw some baby powder in it to make it gray. Then you need a beaker with dry ice.

A4: The dry ice and water is always a big hit!

A5: I think you should get back-rimmed glasses with masking tape in the middle, put your hair in a pony tail (if it's long enough) with some strands of hair pulled out, maybe some mismatched shoes or socks, and a flask full of a questionable substance. Maybe even get some of those costume contacts that make your eyes look red.

A6: Erlenmeyer flask, water, a little dry ice, and a glow stick.

A7: You need to get one or more of those light sticks that glow in the dark. Carefully break one open and sprinkle the contents on a tyvek lab coat or jumpsuit just before the party. i did this to go as a 3-mile island worker many years ago.

A8: Hi Samantha. Over the past 21 years I have resurrected a costume I made out of a standard lab coat. To this I glued on a variety of microbiology disposables, including Petri plates, pipettes, blades (dulled!), pipette tips, gauze, tubing, etc. Then I took some silicone, colored it yellow-green and spread it around everything, making it look like microbial growth. I also glued on a Foley catheter to the rear, as well as some other hospital-related items, and colored these in a nice blood red or green sputum color! When asked what my costume represented, I simply stated, "The Jersey Shore!" I can say that 'cause I'm from Brooklyn! It was a big hit. But my wife said it was time to go. Ah, memories!

Validity of Sterility Test Results

A laboratory that performs sterility testing does not include negative controls in the testing, but instead uses the results of the sterility test on the media when it is prepared. This same laboratory does not perform viable monitoring of their analysts each day that they perform sterility testing, instead analysts are monitored twice a month. The laboratory also uses two analysts, designated as primary analyst, and secondary analyst, to perform sterility testing in a LAFH at the same time. The primary analyst performs the testing, while the secondary analyst moves items in and out of the hood during the testing. Is a positive sterility test result obtained by this laboratory a valid result?

A1: I see a couple of issues with this set-up. If I understand it correctly, the testing is done in a laminar air flow hood. By this I assume that you mean a horizontal sterihood, as a BSC Type II hood is neither laminar flow nor controlled. Although not stated in your Email, I also assume that the interior of the steri-hood is ISO 5 (Class 100) and monitored during the test. I also assume the surrounding area is ISO 6 (or minimum ISO 7) and monitored.

With all that in place, the test is still out of compliance due to the lack of sufficient personnel monitoring. Having said that, if your client is still willing to let the lab do their testing they almost have to live with the results unless they can document a clear event that compromises the test. The time to protest was before the test was done.

A2: My take on this is that a False Positive, caused by environmental or personnel borne contamination is still a Positive (Fail) unless you can convincingly confirm that the bug was indeed from one of these sources, and not actually in the product itself. Without the negative control, or adequate enviro/personnel monitoring you simply haven't got any data against which to compare, so the batch will fail every time.

I note [name redacted] comment that the test is "Out of Compliance", and I would like to discuss that further. I have a different interpretation and I suspect it's to do with the difference in regulatory environments in which we both exist; the FDA are, I'm led to believe, rigorously and incontrovertibly locked in to rigid interpretation of the USP, whereas the TGA interpretation of BP/EP, via the PICS cGMP code (read: "Guidelines"), are a bit more pragmatic and open to dialogue;

The laboratory in question, if regulated by the FDA would, as Scott says, be Out of Compliance, and its ability to perform the test questioned. In PICS regulated parts of the world we are able to discuss this with our auditor and present a case that the sterility test environment "compliance" is, at least to some degree, a business risk, and not wholly a compliance issue. If the business makes a decision to risk it's products' release by cutting corners in Sterility Testing, then as long as it accepts a higher risk of artificially failing a batch, the regulator may tolerate "non-compliance" with the strictest interpretation of the compendia.

This standpoint is the basis for my strong opinion on the use (or misuse) of the Negative Control, and may explain why some (American?) respondents disagree with my interpretation. In my regulatory environment we are not completely hung up on following the compendia to the letter, but can openly discuss and challenge our application of "guidelines"; we can (and do, frequently) go better than the compendia in some aspects of the sterility test, but also we are able to acknowledge that as long as the Sterility test suite is not the manufacturing environment, there is no risk to product batch integrity just because a few discrete samples of a batch get mishandled, or exposed to non-Class A conditions during testing; even though the likelihood that they will fail the test under such "relaxed" conditions is of course greatly heightened, that's a business risk; a fail is still a fail, and a pass, even in Class B, must still be a pass. I have, and will continue to successfully argue this with many and varied auditors and consultants in our PICS regulated AOR, and we all fervently hope, for your (American) sakes, that FDA will expedite joining PICS as soon as poss; it allows for serious debate rather than just big stick regulation.

A3: I will appreciate your opinion on the appropriateness of replacing negative controls in sterility testing with an uninoculated and unopened bottle of sterile medium. It is true that the use of a negative control in sterility testing is to demonstrate the sterility of medium used for the test, but it is also used to rule out false positive test results, because the negative control is exposed to analyst manipulations and aseptic techniques, the testing environmental conditions, testing equipment/articles, and other conditions that the test sample is also exposed to during the test. Also is it standard practice to have a second analyst come in and out of the LAFH, while sterility test is being performed?

A4: I would assume the lab would initiate an investigation, lab would not have any data to prove there is no lab contamination, test environment is not monitored. Do they at least monitor the hood? What about gloves/instruments used to primarily handle the sample?
I can already see the result of this investigation.

A5: I agree with [name redacted], protest should be before the test result is out. I don't understand the need of the second analyst in the sterility suite. The lack of negative control and no daily personnel monitoring ... did the lab even perform environmental monitoring in the sterility testing suite?

Prior to using contract testing lab, we must audit the lab and personnel to ensure compliance and competency.
Did your client protest the lack of compliance and control when sterility test passes?

A6: To be honest I see little value in the required negative control in the Sterility Test. First of all, as has been amply demonstrated in this group discussion, many feel it is appropriate to only use this control when it is in their favor. In my mind this is intellectually dishonest. If the control is of no value when the test passes you are basically admitting that the control is not appropriate for its intended purpose, which I guess is to serve as a check on the technician's technique and the testing conditions.

I actually would prefer to avoid this misleading "control". Better nothing than a poorly designed effort that is routinely ignored. I think that we would be much better off to demand people stand or fall on the basis of the test. If you cannot prove the test invalid, it is valid. Now this opinion may be because I live in an area where the regulatory climate has been characterized as somewhat anal-retentive, or it may be because I believe that you should consistently apply scientific principles (maybe they are not too dissimilar?).

The deeper concern, for me, in this situation is that the client was apparently fine with what appears to be a very poorly controlled environment and lax procedures until the test failed, and only now is concerned about the poor conditions. One of the common problems I see in my consulting practice is the inattention of many companies to the technical capabilities of their contract testing labs. Just because a lab claims to be able to perform microbiology testing does not mean they can do it in a competent fashion. Sending Quality (capital "Q") people out to audit the lab (if an audit is conducted at all) does not allow for a technical audit of the lab's capability. Given the operator-dependent and technique-dependent nature of microbiology, ignoring the technical qualifications for the ISO or GMP documentation considerations common in this audit approach creates unhappy situations like this.

I would urge all who can to insist on accompanying the audit team if micro testing is on the agenda. A good script for the technical audit is provided by PIC/S (PI 023-2 AIDE MEMOIRE ON INSPECTION OF QUALITY CONTROL LABORATORIES) and can be found at http://www.picscheme.org/publication.php?id=14.

Qualifying the contract lab ahead of time can avoid a good deal of trouble (see http://www.microbiologyforum.org/PMFNews/PMFNews.15.05.0905.pdf for a more complete discussion of contract lab qualification).

A6: Great discussion on the negative control. Most people would agree that an experiment needs a positive and negative control. Many times the negative control may be used to help the analyst interpret the results correctly. The negative control is helpful in reading reactions on many selective and differential media. For the sterility test a negative product control (See below definition) would be more useful. Many times the product adds turbidity that is hard to not interpret as growth. A follow up streaking of the broth to agar media is performed. Not without risk.

Question, does the negative control for the sterility test also include the FluidD used?
In this case it is useful to know the fluidD was not contaminated.
I think it is useful to know that the media or fluids used in a test are not contaminated.
I think our system of making material sterile has gotten so good it has invalidated the need for the negative control.

I remember leaving media in a water bath over night, having contaminated negative controls, then realizing the value of the negative control to curb bad practices. That is a bad practice.
Many times people do not believe until they see with their own eyes.

determine the Endotoxin limit(not in Appendix E)

Recently I have facing problem to determine the Endotoxin limit for (not in Appendix E) parental products, for example.

A1: We use the maximum dose from the product insert as M. In case it is in mg/m2/h we convert it to mg/Kg/h using a dosage calculator or considering a 70Kg and 1.8m2 person.

A2: Endotoxin Limit determination of unknown sample depends as per dose of that drugs. I agree with you and EL setting of unknown sample is very difficult specially M -Maximum Human Dose which is changeable. I agree with Carina. You can follow your product insert for setting of M.

Investigating TOC Failures

TOC isn't really my area of expertise but I am investigating some WFI fails which have occurred recently. I am interested to know whether the cause of contamination can be traced/investigated i.e. if it can be determined whether the carbon is from microbial contamination or chemicals (e.g. disinfectants).

A1: In my experience, must TOC events are not related to microbial bioburden in the water but are related to the use of alcohol during the sanitization of the user point. Please consider that if the Microbiology lab does their sampling before the analytical lab, that the alcohol vapors in the atmosphere will interfere and give you high TOC results. The same thing could happen when the sample takes place during or right after manufacturing processes.

I would suggest investigating your microbiological data results for your water system on the date of your event which will help on your search for root causes. In fact, it will take lots of bugs to reflect it in your TOC readings, you would rather first be aware of their presence in your microbiological tests or your endotoxin levels. The microbial content expected for a WFI sample is 10 cfu per 100 mL (maximum action level) but for most WFI water systems these numbers tend to remain very low or at zero.

I think that by just looking at the above suggestions you will probably have enough data to make a good determination without having to do extended and costly characterization tests.

A2: Some years ago, I did a calculation with certain assumptions and found the it would take several orders of magnitude over the normal counts of organisms in water. I agree with Ruth that it is most likely caused by the overzealous use of alcohol, a dirty environment, using powdered gloves, gaskets breaking down in the water system, a failure of the purification system, or any one of a myriad other possibilities. But not micro contamination.

A3: High TOC results can come from a number of sources, including headspace in the vials, if you are using a laboratory unit. High TOC is commonly caused by your source water. It is somewhat difficult to show that microbial contamination relates to higher TOC results, but it can be done.

A4: Sine it is WFI, Microbial Contamination chances are very less. Investigate the status of Sampling port, frequency of sanitization, Cleanliness of sampling bottle, air space in a sampled container, integrity of the sampling container and lid, time between sampling and analysis and sampling practice.

Based on the above mentioned investigation you may conclude the possibilities of TOC failures.

Amino acid sterilization

Does anybody tell me that how or what will be the condition for amino acid sterilization? Is there any method for terminal sterilization?We are going to prepare Amino acid LVP?

A1: In my country few companies are autoclaved their Amino Acid product at 106 deg.C for 85 minutes. Actually there is no reference guide for this temperature. In my last company produce this product and they follow terminal sterilization at above temperature and time. I validated this temperature by self contained Biological Indicator-Bacillus stearothermophilus -6 Log and log reduction is 6.

So I think that you can try those process and may be successful.

Effect on KTA test result for Onset Value

In KTA method there is a option to change Onset value. I want to know that have any effect for resetting of onset value in test result that means Exact endotoxin value?

A1: In theory, changing your onset time should increase or decrease the time of your assay reaction and change slope and y-intercept values. For example, if you make your onset time shorter (smaller), you would expect a curve with more slope and higher Y-intercept values.

Depending on how far your raw result is from the mid-point of the curve (assuming you spiked there), you might see differences in an endotoxin result.

Now the reality is I've tried this one time with Endosafe Endochrome-K chromogenic reagents using a spike of 0.5 EU/mL and didn't really observe a difference by decreasing onset times by 100 mOD. I didn't have activity in my product and I haven't included PPC recoveries, however there was no noticeable difference in %PPC recovery.

Generally speaking, you should use the system parameters specified by the reagent manufacturer.

delta (mOD) 200
correlation -0.997
slope -0.209
y-intercept 2.865

delta (mOD) 100
correlation -0.995
slope -0.206
y-intercept 2.838

Biological Assay Method

In BP or USP are stated that two method of Biological Assay can be followed such as Cylinder Plate-CP Method and Turbidimetric Method. Most of the companies follow Latin square Plate Design Ager Diffusion system. I want to know that is Latin Square plate design system called Cylinder Plate Method ? Recently my internal auditor raised a NCR on Biological Assay Method, where I mentioned in my method as "Agar Diffusion Method" and the auditor told me that in BP or USP there is no mentioned any method as Agar Diffusion Method. Am I right or wrong ? Can anyone share with me ?

A1: USP General Chapter 81 'Antibiotics-Microbial Assays' provides option of using Cylinder-plate or Turbidimetric. In my opinion Turbidimetric assay is better than the cylinder-plate method.

A2: Cylinder plate method is the same as you call Diffusion agar method. You can tell the auditor to read both methods, yours and USP/BP and see they are the same. Its only a problem of title.

ultra filteration

Can any one clarify the method of testing ultra filtration? How to conduct the challenge test.

Classification of BSC-containing rooms

For a company obliged to be compliant with the European Pharmacopeia Annex I in terms of its environmental viable and non-viable regulatory guidelines, what is the “industry standard” in terms of setting action/alert/excursion limits for class 10,000 (ISO 7) rooms which house Class 100, Biological Safety Cabinets ?

Does anybody follow the draft USP(1116) Feb 1997 document which is more stringent than either EU or US FDA Guidelines?

A1: You should monitor your ISO-7 background using appropriate alert/action levels based on ISO 14644 and historical data. You should do the same for your ISO-5 BSC. In regards to USP <1116>, there is a newer version than 1997 that has incorporated the ISO limits.

Sterility Testing of In-process samples

What is the industry practice of sterility testing of the in-process samples like vials, stoppers and the seals. Is it only done during hte process validation studies or during the routine batches too? The controls on the processes(depyrogenation/ sterilization) that render these materials sterile are being reviewed batch to batch basis and actions taken if not meeting the validated parameters. Even after these controls, do we need to sterility test the samples like vials, stoppers and seals for every batch? What is the best industrial practices? Are there any norms(regulation or guidance) on this activity?

A1: In process sterility checks to be performed for each batch what we have manufacturing.
these checks plays a main role when sterility failures occur. In process checks to be done for leftover glass vials, stoppers and seals which are remaining after the completion of batch filling and Leftover filtered Bulk samples to be consider for sterility testing. Before starting of filling and after completion of filling compressed air samples to be collected at filling area to perform the sterility testing.

GPT with Isolates

This whole story of growth promoting the media with the environmental isolates is of no value addition. After all, the isolates are obtained using the same media. Then what is it we are proving by GP testing the same media once again (with the isolates obtained using the same media)? But this is a favorite of all who audit the pharmaceutical facilities.

Performance Qualification of Autoclave

I have some questions concerning Autoclaves:

1.Is there any international standard that determines the acceptable criteria for autoclaving at 121 °C ...I mean whether 121 ± 1 °C..or different?

2.is it acceptable that the PQ (performance qualification) shows a reading< 121°C at anytime....if yes: is there any acceptable criteria for it?

A1: yes
iso 11133-1,2/2009

A2: 1. Temperature acceptance criteria for autoclave is 121 +3 °C. It should not be in minus. Further clarification ref HTM2010 guidelines.u can download it from google.

A3:
1. USP clearly speaks about autoclaving different (almost all) media at 121 C for 15 mins, as to avoid the media charing. But for other articles like glasswares and SS tools many companies run the autoclave at higher temp for lower time (e. g. 134 C for 4-5 mins) duration as per the initial qualification studies.

Ultimately it depends on the load you are going to sterilize and accordingly the qualification data established.

2. In PQ, there can be a drift in one of the thermocouple (say in 1 out of 12 thermocouples) during the hold period, but the acceptance criteria should be in line with the regulatory requirements i. e. the F0 value should be 15 minutes and the SAL should be 6-log reduction of the biological indicator (That thermocouple should be coupled with the biological indicator). If these are fulfilled then the instance is justifiable.

isolators (class A) air monitoring

Regarding air monitoring within isolators (class A, sterile API manufacturing) we have tried both settle plates and active air sampling by means of an external Millipore pump (M AirT isolator pump). We are worried about contaminating isolators environment due to the introduction of culture media inside. Does anyone know a different way for isolator monitoring without putting in risk its sterile environment?

A1: Please forgive the commercial nature of this posting, but Sartorius Stedim Biotech supplies gelatin membranes for use with our MD8 series of active microbial air sampling devices, which eliminate the introduction of growth media into your critical environment. The gelatin membranes collect airborne microbes through a combination of filtration and adsorption mechanisms while also maintaining the hydration of the captured microorganisms. This prevents the desiccation of microbes as seen with traditional membrane filters, such as cellulose nitrate. The gelatin membranes do not support the growth of microorganisms on their own. To get the microorganisms to grow, you simply transfer the gelatin membranes to your choice of solid growth media (agar plates) or you can also dissolve the membranes (they are water soluble) for a variety of alternative applications to recover or detect the microbes.

A2: We offer a remote sampler, that allows sampling close to the critical process using an isokinetic probe, while the media plate is located a bit further away from the critical location. The plate however is still inside the isolator. A benefit is that the unit can be fully autoclaved or gaseous decontaminated and that it does not disrupt your laminar airflow. The standard media it uses are sterile and the unit allows for longer sampling times - meaning fewer interventions.

Placing the media outside the isolator has two major objections. One reason is the higher risk of false positives, since the background of an isolator is normally a grade D. And secondly, the tubing going out of the isolator might easily get too long, with the risk of losing larger particles inside the tubing. A well described problem in non viable particle counting and a concern for the agencies. Next to that you have to somehow decide how you want to deal with the gaseous decontamination.

A3: Isolator users have been using settle plates and active air monitoring (SMA's, etc.) for many years and I have not heard of any failures in germ-free maintenance of the enclosures due to their use. There has been concern expressed on the use of RODAC plates for surface monitoring because of media residues, but a properly applied cleaning step should keep the risk at bay. I would recommend that you continue your current practice of isolator monitoring.

A4: The media plates you should be using are radiation sterilized in sealed single or multi-layer packages made from a VHP resistant wrap. A number of manufacturers make these types of plates.

If you choose to use the multi-layer wrap, remove one of the packaging layers just prior to the decontamination cycle; this will lower the bioload prior to decontamination. You still have to make minimize the surface-to-surface contact and place the wrapped plates on wire racks to expose as much surface to the VHP as possible. Adjust/flip the plates mid-exposure to expose the wrap surfaces in contact with the wire rack.

Isolator for sterility testing

My laboratory is thinking of buying an isolator for sterility testing. We have two offers, one is from SKAN (Isolator PSI-M) and the other is from LA CALHENE (Isolator ISOTEST). We are thinking which one to chose. If someone has one of these kinds of isolator, would you mind to share your experiences with me?

A1: From the few experience I have with La Calhene Isotest system, apart from the laminar air flow in the Skan vs turbulent in the Isotest system I will say the 2 systems are more or less equivalent. The software is quite basic 'though and you will have to be very specific in your URS. Some "standard functions" (or what is generally considered as industry standard) are not included in the basic model, but these are minor issues that can easily be resolved.

Where the big difference lies is the experience and the technical support provided by the supplier/ manufacturer and again from my own personal experience, I will say that La Calhene still have some way to go compared to SKAN.

You can go ahead with the ISOTEST system if you have an experienced in-house technical team.

Microbiology

We received a FDA microbiology deficiency as noted below for a drug product.

"Please provide data demonstrating that the diluted drug product does not promote microbial growth under the worst case dilution and storage conditions, for the drug product (i.e. 24 hours at room temperature in 5 % Dextrose and 0.9 % sodium chloride injection). Please note that the RLD may be tested in parallel to demonstrate equivalence."

As per my view, the drug product (vial) must be reconstituted into the diluent (I.V bag) under aseptic conditions, and perform a sterility test after leaving it for 24 hours at room temperature demonstrating that sterility is maintained after the intended time and dilution exercise. But one thing is unclear to me to perform this.

When we inject the drug product in to the plastic I.V bag (diluent) the integrity of the sealed bag is lost, as there is a breach when we inject the drug product into the diluent bag, so how to perform this without losing the integrity of the I.V bag?

Is there any alternative approach to demonstrate the same?

A1: To the best of my knowledge, there is a test, very similar to the preservative efficacy test, that you can perform. I believe this test is listed under the ISO Guidelines, but I am not sure as I am retired, and don't have the reference materials necessary.

But in essence, you prepare the product so that it is at its use-dilution, then remove aliquots of product and challenge them with 10E5/mL of the five test organisms from the preservative test (individually). You then plate out the challenged aliquots every so often, for example at 6, 24 and 48 hours after storage at 20-25C, and if the challenged product does not show an increase of more than 0.5 logs at 24 hours, you have proven that it is OK to keep the reconstituted product for up to 24 hours.

It is a test that is used for non-preserved multi-dose products.

A2: There is a way you can seal the tubing leading from the injection ports to the bag itself. You need a pair of pliers (sterile of course) :) and metal loops/clamps. Using the pliers squeeze on the metal loop/ clamp to seal off the tubing. There are also plastic tops that you can place on top of the injection port itself they just snap on and they are very hard to get off :) it has been a while since I have had to use any of these so I am not sure where to buy them from.

A3: PLEASE TRY USING THE DILUENT SOLUTION PREPARED IN YOUR LABORATORY WITH PROPER CONTROLS IN STERILE GLASS CONTAINERS.

THIS SHOULD SOLVE YOUR PROBLEM

A4: Please explain "RLD may be tested in parallel to demonstrate equivalence. what exactly this means to say.

Regarding SCDM GPT using anaerobic organism

AS per the PIC's Guidelines the SCDM should be subjected to GPT using anaerobic organism (Clostridium sporegenes),kindly let me know

1) whether it is mandatory to perform the GPT

2) Incase if we do will any growth occurs and how it would be(i mean the growth pattern).

Based on our experiment i did't observe any growth within 48 hrs of incubation period in anaerobic condition (inside theAnaerobic jar).

A1: The section in PIC/S Guideline suggesting that an anaerobe be used for GPT SCDM is misleading.

I contacted the organization requesting that it be revised so labs will not try to do this test and auditors will not request that it be done.

A2: As per the USP or any other pharmacopeias, TSB or SCDM does not include anaerobic organisms (see sterility testing chapter). You are right fastidious microorganisms or anaerobic microorganisms do not grow on SCDA or in SCDM. As you know that small amount of agar favors the growth of anaerobes. L-cystine and Sodium thioglycollate helps anaerobic bacteria to grow even in aerobic condition because Sodium thioglycollate helps to neutralize the toxic effects of mercuric compounds (if present) and both of them collectively act as reducing agents and expel oxygen from the medium to neutralize the peroxides formed in the medium, thereby promoting anaerobiosis. For these good reason Fluid thioglycollate is supposed to good medium for growing C. sporogenes. To help you I can suggest you to put 1% Glucose in SCDA or in SCDM that will help C. sporogenes to grow. OR to confirm that C. sporogenes show good growth u can add agar in Fluid thioglycollate medium to solidify it and then put the culture and incubate the plates in anerobic jar. This is the experiment I did and I found good growth of C. sporogenes.

A3: Similarly i have one more query,

whether anaerobic personnel monitoring is required during media fills, i mean the one more set of RODAC plates need to be used in the same locations where we perform the regular personnel monitoring and that need to be incubated under anaerobic condition to check for chance for any anaerobes.

Is there any reference or guidelines available for that.

A4: Anaerobic personnel monitoring is not a requirement in the FDA aseptic processing guideline, EU GMPs Annex 1 or USP <1116>.

raw material

I have a question related with a Raw material, which is know done by enzymatic synthesis. Following the monograph it is not required de micro testing but would it be necessary know that the way of synthesis has changed and it's biosynthesis.

i want to know the limits for oral product containing additives which are of natural extract like rhasberry my confusion originate from it is additives and not active substance.

A1: You have mentioned that your product contains natural origin Rushberry. I think that your product is categorized as Oral Preparation, the limit as below :

Total aerobic count not more than 1000 cfu/ml for bacteria and 100 yeast and mould/ ml.

B. *E. coli* must be nil. Ref. BP 2009 - Chapter -Pharmaceutical Microbiological Quality Control

It may be helpful for setting of limit of your product.

microbial limits in Europe

A question mainly directed to Cosmetics Microbiologists releasing products
in Europe.
Do you guys release based on
1) limits as specified in Eur Pharmacopeia (Max 200CFU/ml Bact, 20CFU/ml
Yeast/Mold)
2) on Annex 8 of European Commission Guidelines for Cosmetic Product which
states very briefly Category 2 products (all that's not baby, or eye/
mucous membrane) can have 10^3 CFU/ml.

A1: I test according to 2) on Annex 8 of European Commission Guidelines for Cosmetic Product which states very briefly Category 2 products (all that's not baby, or eye/ mucous membrane) can have 10^3 CFU/ml.

A2: The microbial test specifications indicated in the European Pharmacopeia are for drug products. Most cosmetics in Europe are not drug products. Unlike fluoride toothpastes and sunscreen products which are drug products in the USA, they are considered to be cosmetics in Europe. As far as Annex 8 for the European Commission Guidelines for Cosmetic Products, it is my opinion that the 10e3 CFU/gram specification for non baby or eye/mucous membrane products is the minimum microbial test specification for count. However, you will find that most cosmetic companies are using far more stringent microbial test specifications for these types of products than what is found in Annex 8 or in the CTFA Microbiology Guidelines. These more stringent microbial test specifications are usually based upon a risk assessment that had been conducted to determine what is acceptable microbial load for the product itself and/or to the consumer. If you conduct microbial analysis of various cosmetic product formulations, you will find in most cases that they have a microbial load of less than 10 CFU/gram. This microbial bioburden level can be easily obtained if you have the proper controls in place for raw material quality, manufacturing equipment and cleaning/sanitization of the manufacturing equipment.

A3: Cosmetics should be tested and released as per CTFA guidelines.

I have 2007 revision that stated that:

Baby Products and Eye area products: no more than 10^2 cfu/g or mL
All other products - not more than 10^3 cfu/g or mL

10^2 - maximum limit of acceptance is 5 x 10^2
10^3 - maximum limit of acceptance is 5 x 10^3

Transfer from TSB to Selective media

it is necessary to transfer a suitable amount of TSB to selective media if not growth in TSB?

if RVS and MacConkey broth don't have growth, it is necessary to transfer to selective agar ?

A1: How you know that the RVS don't have growth??? The growth in RSV is not indicative as MacConkey broth. Anyway yes, you must transfer to selective media.

A2: I can show you a Pseudomonas in TSB solution after 18-20 hours that doesn't show growth, but when you plate it on cetrimide (even after less time than 18 hours in TSB) it'll show a plate full of colonies. Always allow to grow in TSB for at least the minimum period of time and always plate on selective plates.

A3: Agree that plating of enrichment is important but also be aware that there are data reporting plenty of pseudomonads, including isolates of aeruginosa, that do not grow on cetrimide. Additionally, some strains of enterics that do grow on Cetrimide (esp Proteus, Enterobacter and Serratia spp.).
So use caution in depending exclusively on this medium.

For example:

J Clin Microbiol. 1988 September; 26(9): 1910-1912.

PMCID: PMC266749

New selective medium for Pseudomonas aeruginosa with phenanthroline and 9-chloro-9-[4-(diethylamino)phenyl]-9,10-dihydro-10- phenylacridine hydrochloride (C-390).
M E Campbell, S W Farmer, and D P Speert
Department of Pediatrics, University of British Columbia, Vancouver, Canada.

Testing of Compressed Air and Room Monitoring

I have a couple of questions for those who are involved in room monitoring and compressed air testing of Non-Sterile facilities. I welcome any comments the Forum may have since someone may have already dealt with similar issues. My questions are:

1) Compressed Air: A) What is the normal classification used for compressed air in a non-sterile, solid dosage facility (product contact)?
B) If the air compressors in use are oil-less, can the test for oil mist be omitted?
C) What is the frequency of testing?
D) Does Nitrogen need to be tested for particulates, water vapor, oil mist, etc. at specific use points (product contact)?

2) Room Monitoring: Hypothetical situation: You have a manufacturing room and a room used to package semi-solid product. This area does not get used frequently. The area has been qualified at a specific air flow (air exchanges) and temperature, but the validation specifies air flow and temperature ranges. The question has come up to reduce the number of air exchanges and to raise the temperature (within the "validated ranges") during times of inactivity. What should be done in the way of room evaluation; i.e., particulate counts, viable counts, etc. prior to the reduction and during the reduced state? What needs to be done when preparing to bring the room back up; i.e., room qualification to show that the present conditions are the same as before going into a reduced state and show that the room cleaning was effective?

A1: 1 A. In a non-sterile solid dosage facility, there are equipments that use compressed air and have direct contact with the product, these user points are classified as "product contact" user points and will require microbial monitoring.

1 B. If the system is oil-less then you should be able to justify omitting the test. The key part is documenting your rationale based on the equipment design and the overall compressed air generation system. As part of your rationale you should provide all supporting data from the manufacturer.

1 C. While I was working at solid dosage facility, all product contact user points were tested twice per year, that is, once per semester. Because we were under a consent decree, we were exhaustively audited and found always in compliance.

1 D. I would guess that it all depends upon the use of the Nitrogen but I am not familiar with these requirements.

2. Is the AHU use at this room also used at other rooms? Could the changes in flow rate affect other areas? My answer would depend on what was done during qualification. If no microbial data was obtained during this qualification then data gathering is a must to understand if the room maintains its qualified state such as temperature, humidity, viable and non viable particle testing. For viable monitoring I would do non product contact surface sampling (facility and equipment) and air. After room cleaning and before startup I would monitor same locations to show cleaning effectiveness plus equipment product contact surfaces.

A2: Dear steve please could you elaborate your question a bit more, we have done the oil less compressed air monitoring just recently, it would be my pleasure to share my experience with you.

A3: I also have a couple of questions concerning compressed air monitoring.

1. Are non sterile facilities monitoring every validation use point?
2. If multiple use points are present after the filter - is each use point tested or just one after the filter?
3. What constitutes a "validated" use point? We considered a validated use point after 3 consecutive samplings resulting in acceptable results. Just wondering how other facilities are handling.
4. Sampling frequency???

A4: We are a non sterile facility, and we do test each validated use point which has product contact. If the supply to the room/area is split into two outlets, they are after a filter. We just test one of the two points. We consider a use point validated when the following occurs: If a use point is placed into a room that didn't have a "drop" previously or when new construction has occurred and a use point is included. The Validation Group issues a protocol to test the compressed air line. You could call this a "qualification" but they consider it validated if it meets the specifications they set. The specifications are taken from the ISO Standards. We test twice a year.

I hope that this helps, especially since the non sterile facility doesn't have any guidelines per se.

Query on media storage

Please if I can Get some answers as to, at what temperatures Prepared Media Plates can be stored and for what duration? Is it only at 4 to 8 C and Is storage at 20 C alright for a week?

A1: Holding time and temperature (20-25°C or 4 -8°C) shall be validated. 20-25°C is Better for prepared media plates.

A2: We store the prepared plates for a month in the fridge (4-8 ºC) but you have to validate the storage time by performing growth promotion tests and negative tests periodically. We've done it with all the media prepared in the lab and it depends on the media but generally a month is good for plates in the fridge.

A3: You can store the plates at 4-8 °c for a week or a fortnight.
What all you need to do is to validate the storage time period along with storage condition.
You have to pre-incubate the Prepared media plates and then store it at 4-8 °c.
You need to check the GPT and sterility of the medium at definite period of time till your study is continued.

Also you have to check the physical appearance of the culture media (colour),shrinkage of the medium within the plate(volume) and pH.
If all these conditions get qualified then u can store the media at 4-8 °c.
If you have justified Protocol and Report for this activity, no more query you'll get on this.

Also storage at 20°C can be justified with all these parameters, but I don't think for prepared plates this temperature condition would help you.

A4: 1. If media plates are purchased, these should be stored as per manufacturer instructions.

2. If the media plates are prepared in house, they should be stored in sealed packages and as per validated condition. I have seen storage of "in house" prepared plates at 20°C to 25°C for two weeks but the practice was discontinued because these were prone to contamination and would dehydrate quite rapidly.

Storage time at a higher temperature followed by an incubation period at an even higher temperature could lead to dehydrated plates where microbial recovery is affected.

A5: The manufacturer may have some guidance. I am sure you are referring to media you prepare from DCM and then autoclave and store. Perform a Prepared Media Stability Study to determine shelf life per type of media. Pull samples at defined intervals, test for growth promotion (and sterility check if you desire) to see that it meets requirements. See how far out you can go. This establishes your expiration date. In an ideal world, it would be great to store some at as close to 2 degrees as possible, some at as close to 8 degrees as possible and prove that this range is acceptable, although it is common industry standard to accept 2-8 degrees as equivalent refrigerated storage condition. It just makes your argument for media shelflife all the more compelling. Make sure you include all temperature monitoring results with the final report. Simulated unplanned excursions/spikes may even be of value, but its best to just have well controlled equipment and not bother and if needed discard media potentially impacted by any future excursion.

A6: Prepared media plate should be store at 20 - 25C for 48 hours this is general practice as per USP and for further storage there must be perform self life of prepared media. Based on study of self life of media u can extend days.

A7: Right in house prepared media plates can be stored at 20-25deg cel for 48hrs but if you are sourcing these plates from manufacturer then you can store these plates at recommended temp for that duration of time as expiry of plates.

A8: Can anyone tell me how to check the pH of solidified agar medium plates after long storage in refrigerator?

A9: You can use a flat head glass electrode. Speed of response at lower temperature might not be that good so make sure your agar is equilibrated to around 25 degree centigrade.

A10: There are flat pH probes available for checking the pH of media.

A11: You have to use the PH meter with a flat electrode for checking the solidified agar medium plates.

A12: If holding time study is not performed, Solidified agar medium plates stored for long time in refrigerator should not used for any testing.

A13: Is there any distributor known in India who can provide us with this product. and what about the contamination chances after the sterilization of media?

A14: You have to hold time study; in you have to periodically check the GPT., The recovery should be meet as per regulatory requirement.

Determination of Fiducial Limit of Error

I several times raised a query regarding Fiducial Limit of error determination. Nobody replied me. I think maximum microbiologist does not know how to calculate the fiducial limit of errror of Microbiology Assay. In BP and USP are stated for that issue. I did not understand anything. I use two dose for a sample- High Dose and Low Dose - Agar Diffusion Method. How will I calculate ? Have any reference guideline for fiducial limit of error?

A1: Kind of a bit harsh on our micro colleagues don't you think? I am sure we can find little Einstiens that do wonders with numbers even amongst US!... any way I think you have not heard much regarding the "Fiducial Limit" because it may not have been utilized often. This may have to do I guess the controversy regarding its ability to predict probability. I am sure there is a way to do what you want to do with out diving in to fancy mathematics. Now what is it you are trying to prove?? Are you trying to determine confidence intervals for your agar diffusion assay. What is the assay? Is the relationship between your concentration and observed response Logarithmic?

A2: In pharmacopoeia is stated that for few Antibiotics those specified of Microbial Assay, Fiducial Limit of error is not less than 95 percent and Not more than 105 percent. I actually asked for determination of fudicial limit of error. Agar Diffusion Method is followed in My Lab. Two dose High and Low for standard and Sample are applied in Latin Square Plate - 8 x 8.

Example : Zone of Inhibition -mm
TH- 23.09 mm Test High
TL- 19.89 mm Test Low
SH- 23.05 mm Standard High
SL- 19.79 mm Standard Low

High dose - 40 IU/ml
Low dose - 20 IU/ml

Sample wt. taken -1.145 g -4000 IU Neomycin equivalent > 100 ml
Standard wt. taken 61.09 mg - 40000 IU Neomycin equivalent ....> 100 ml .... 5 ml......> 50 ml

A3: Most of microbiologist is weak to calculate Fiducial Limit of error of Microbial Assay. This has reflected in poor reply in the issue. OK. I already calculated this.

A4: I am glad you solved the problem. I do agree with you that many of us may be "mathematically challenged". Descriptive statistics is what we mostly deal with and our field generally is descriptive. However, One should not have much excuse for not grasping basic tools if one is into science. Any way If I may share my USP multiple plate assay experience...,

The confidence limit (I prefer to stick to this term rather than fiducial limit) is determined from log values of antibiotic potencies (Chapter <111> Design and analysis of Biological assays. Equation 24 and 25 are the one's that we should use. Equations 24 gives you the log values that you add or subtract from the mean log potency to get the upper and lower limits of potency at 95% level of confidence. You can then compare if these limits meet your % of potency value criteria.

Alternatively (and I have discussed this with USP a couple of years ago) you can use a simpler approach. In this approach you convert potency values into log values (does not matter what base) and calculate SEM (standard error of the mean= SD/sqrtN). Your lower and upper limit of the confidence interval will be mean +/- (1/2t*SEM). 1/2t = value you read off a T-distribution table at the degree of freedom of your sample (n-1), at probability chosen (if 95% confidence is required a probability(t) 0.05, but you read of at p of 0.025 /one tail is used. Once you get the log figures find the anti log to express in concentration unit. This should give an asymmetric upper and lower limit ranges around the mean at a desired confidence limit. The same approach can probably be used for data generated by single plate/Latin square setups.

Hope at least some of the faith in our kind restored!

Laboratory environment for BI set-up

I would like to canvas the group on the laboratory environmental conditions used for setting up BIs. We set them up in a Biosafety cabinet that is located in Class D lab that we thought was satisfactory until an auditor suggested they be set up in Class A cleanroom. We would like to gauge what industry practice is?

A1: We set them up in a BSC (ISO 5 particulate) that is located in an uncontrolled lab space.

A2: That is funny because BIs are made in a Grade D room with not much concern for sterility. The risk of contamination is very low. Not many organisms can grow at 55C. Most contamination comes from the positive control not being tested as the last sample.
Why would you want to bring live spores in a grade A cleanroom?
A BSC in an uncontrolled area is industry standard. The Microbiology labs are usually not in a class 100,000 area.
Europe may be different.

A3: Hi Paul. Does your biosafety cabinet meet Grade A expectations? If yes, that should be sufficient. However, I have to ask what you are using your BIs for. That may explain your auditor's suggestion.

A4: I'd like to meet that auditor (I guess I may have already!?). Who would seriously consider taking live inoculae into a Class A? Particularly a spore? What sort of precedent does it set for those of us who regularly use BIs to validate autoclaves in businesses that do not have , nor need, a Class A facility? Your set up sounds fine, and the lab, being presumably PC2, is equipped and staffed with resources more than adequate to manage safe aseptic handling of BIs. Sounds like a non-microbiologist auditor, with little or no sterile manufacturing experience... I love being audited by them!

A5: We process our BI's in a Biosafety cabinet that is located in an unclassified area, I would have thought that it was the classification of the area that you are processing the BI's that was important, ie. the classification within the safety cabinet.

A6: What strikes me as even odder is that we are treating a BSC as if it were a controlled environment. The point of the BSC is, of course, to provide a cabinet wherein we can manipulate pathogenic organisms (in Type II BSC - risk level 2 organisms in operations generating aerosols) in relative biologic safety. It is not designed nor intended to be a controlled environment. Fortunately, it is a relatively small and enclosed space so we are able to sanitize it sufficiently to meet cleanliness standards despite its inappropriate design for that function.

If there is a reason to perform BI prep in a BSC it is not clear to me, but much of what we do is a bit difficult for me to rationalize at times. We should not be concerned about rampant contamination of the BI prep altering the D-value of the sample run as even if the prep was contaminated by millions of organisms on the bench as it is unlikely that the contaminating organism's D-values will be sufficiently large to affect the outcome, or (as you note below) that the organism will be able to grow at 55C. This is aside from the obvious issue that a proficient lab should not be plagued with contamination issues from work done on the open bench. While very few are, it has become "GMP" to perform work in BSC despite its expense in the belief that this practice will minimize the numbers and extent of investigations into aberrant results.

However, leaving the BSC rant aside for the moment.

If I read this thread correctly the inspector wants BI preparation (set-up) to occur in a Class A environment. The suggestion that we should perform "dirty" operations (BI prep has to count as "dirty"!) in a Class A environment should disqualify the speaker from further technical responsibilities in this field.

A7: We also set up the BI's in a laminar flow hood located in an unclassified laboratory. The hoods and personnel gloves are monitored for viable contamination after the testing session.

A8: In a previous life (I have many!), we processed BIs in a BSC for the following reasons:

1. Control of contamination during the transfer of BIs into tubes containing media (see disclaimer below).
2. Control of aerosols when enumerating BIs due to vortexing, dilutions and plating

You should understand point number 2 above. Here is the disclaimer for point number 1:

When transferring BIs from a package or envelope or other container <outside of a BSC>, if you use appropriate aseptic technique, there is little or no chance of any remaining viable spores jumping off the BI and onto the lab bench or into the air. Additionally, if the BI is used to monitor moist heat steam processes, there is little chance that the normal flora in the lab will gain entrance into the BI test tube and grow at 55 degrees C. However, if the BI is used to monitor other sterilization processes, such as EtO, then this may be more of a concern as the incubating temperatures will be lower. That being said, the REAL reason why we performed ALL BI work in a BSC was to keep the chemists and engineers off our backs because any failed BI could have been thought of as lab techs contaminating the samples!

So, to come back to the original post, if you have a BSC for the purpose of processing BIs, use it! This can be your Grade A environment, and everyone's happy. If not, I would push back on the "recommendation" as no one in their right mind would bring BIs into a Grade A environment for processing.

A9: As we are operating a service lab where we routinely perform BI corroboration, but also inoculation of test pieces with BI suspensions, I think I should give my comments to that discussion.

This is a typical risk question. You should be clear about the rationale for you are doing. What is the intended outcome of your operation? The answer to that question and the risks you have to meet your goal decide the measures you have to take.

You want to generate a series of BI to be used for validation of a sterilization process? Your concern is Quality of your BI.

Then you are inoculating a test piece or a product with a very high number (typically 10E6) of viable spores which are typically more resistant than anything you see in a typical lab environment. (Whenever we tested the sterilization resistance of spore preparations from environmental isolates we have never seen a D-value higher than 0.5 minutes.) So, if you contaminate your test piece from the environment you can only expect a negligible number of contaminants (resulting e.g. in 1000100 instead of 1 000100 cfu!) with low resistance. So why shoud anybody worry about contamination? From a scientific viewpoint you could work in an unclassified reasonably clean environment, the error would still be far too small to detect (it is a good exercise to do the calculations!).

You want to transfer exposed BIs to growth medium to evaluate if there are survivors? Your concern is absence of growth in tubes with negative BI units.

Now your situation is different. If you are using G.stearothermophilus, you incubate at 55°C and therefore, most contaminating microorganisms would not be able to grow. Still it is a good practice to use a high quality environment (typically a LF-bench would be used). If you are using B. atrophaeus, you incubate at 35°C, and now you need to work very carefully as each contaminant could mess up your result. A class 100 work-bench would be your minimum requirement. (It is our practice to do verification on each positive BI assay to make at least sure we have grown Gram positive rods of typical appearance, in critical cases full we propose full identification of the BI organisms).

You want to protect your lab from contamination with resistant spore formers? Your concern is release of spore formers to the environment.

The risk is negligible where you only transfer exposed BI to medium. You expect to never see a resistant spore, except in rare cases, and then the spore would probably be severely stressed and need medium for resuscitation. The risk is significant of course where you work with high concentrations of spores to inoculate test pieces or product. You can use a vertical LF-bench (not necessarily a BSC) but it must not be a horizontal LF. It is good practice to work very carefully and avoid formation of aerosols. In addition, my recommendation is to disinfect with a sporicidal disinfectant after every work session with spores. It is also good practice to monitor the lab (air and surfaces) frequently for contamination with spore formers.

You want to protect your personnel from contamination with resistant spore formers? Your concern is release of spore formers to the environment and infection of your personnel.

For release of spores, the same considerations apply as for protection on the lab. With regard to infection of people. neither G.stearothermophilus nor B. atrophaeus are recognized as being pathogens or even opportunistic pathogens. You should bear in mind, that on a dry summer day with dusty air the exposure to spore forming microorganisms from the soil is high to everybody who ventures outside.

In our own lab we are working in a bench under Grade A air supply, to protect our lab and to avoid contamination in transfers. In 10 years of intensive work with BIs we have never seen a massive lab contamination in our monitoring. In transfer of exposed BIs with B. atrophaeus the occasional contamination is seen, and therefore it is mandatory to verify that the growth is due to the BI organism.

A10: Thank you for your thoughtful reply, and yes, I for one was having too much fun with this question.

Having said that, there is a concern (at least on my end) from a query asking if it is now industry practice to "set up" BIs in Grade A environments. I interpreted this as bringing viable spore preparations into a Grade A environment (thereby compromising the state of control of that environment) merely to minimize extraneous contamination that you correctly point out will not be detectable in any event.

If the question is recovery of survivors after a sterilization cycle, I would also argue that this is up to the lab, not the inspector, as the risk here is for a false positive (although use of an isolator might not be appropriate if the concern over false positives is high from the company).

Now you introduce a new topic - the protection of the workers and the lab from contamination as a result of working with spores. This is, in my opinion, the best argument for use of a BSC in this application.

It would be nice to confirm exactly what the inspector's position was.

endotoxin problem

i am working in a infusion (Saline) plant. in my company main raw materials is water & i have a problem which is endotoxin. i test my distillation plant water (WFI) everyday four times & i found different result in LAL test. some times WFI pass in LAL test & some times it fail in LAL test in a day. its mainly found in different multi column in my distillation plant.so please give me some advice.

A1: Actually Distillation process can not be contaminated with endotoxin or microorganisms. Multicolumn distillation is a safe process for removal of endotoxin from water. I think that Your endotoxin contamination may be occurred after distillation where you preserve Distilled water for regular use. Distilled water should be preserved at 85 degree celsius continuously or freshly prepared water should be used. Whole water of reserve tank may be contaminated with the rest part of water. Temperature should be monitored regularly even at holiday. Reserve tank should be sanitized with appropriate disinfectant and you have to be validated these effective sanitizing procedure that can be removed any contaminants from water. Large volume water preservation is very tough to control. Test is not only dependable criteria to control the water system. Test is only part of monitoring. You have to find out the source of contamination of Gram Negative Bacteria where come from of endotoxin. Firstly you have to collect the sample from different point for both test microbial count and endotoxin test as below :

1. Just after Distillation plant
2. Before entrance of Reserve Tank.
3. After reserve tank
4. User Point

Gram Negative Bacteria is the main source of endotoxin. From the above sampling point or any other sampling point that you should determine where comes from contamination.

ISO 14644-1, -2, -3 and ISO 21501-4:2007

Are people using the ISO 21501-4:2007 for particle counters? It is the method for calibrating them on particle size detection.

Does anyone know when the revised ISO 14644-1, -2, -3 may be out? Has anyone seen the proposed revisions?
The proposed revisions are extensive and increase the number of particle sites significantly.

Setting Endotoxin Limit for Combination Product (drug/device)

We are developing a terminally (gamma) sterilized, subcutaneous implant containing a target 50% wt of a drug product intended for a 30-day release of the drug product after implantation at ~150ug/hr. Since this is a combination product (drug/device), I working on calculating an endotoxin limit. With that, will the specification for endotoxin be based solely on the drug itself as calculated per USP <85>? Or is it necessary to also account for endotoxin contributed by the device in the overall drug/device endotoxin limit?

A1: I've never worked with devices, but what we do with injectable preparations which have excipients is consider the API dose and set the limit according to that. Then, as the test is carried out on the whole product the result involves the excipients contribution.

A2: I would follow USP 85 for the drug product. Therefore the drug product would meet compendial requirements. Additional, the device must meet FDA guidelines : 20 EU/device.

A3: Calculate the endotoxin limit for the drug and compare it to the limit for the device. Going with the lower of the two limits ensures meeting the most strict of the limitations for either component of the combination product. If the combination product is tested for endotoxin in final form, the endotoxin contribution from both the device and the drug will be taken into account.

5 micron particle testing in ISO Class 8 (Grade D)

Does anyone set a 5 micron particle limit in a ISO Class 8 or Grade D or Grade C areas?
ISO does have 5 micron particle specs for ISO Class 8.

I have not seen anyone set limits for 5 micron particles during monitoring of Grade D areas.
I did see the EU says may be potentially useful in Grade C areas.
The EU does say, " in Grade A and B areas the 5 micron particles take on a particular significances".

I will be validating some rooms to Grade D and people want me to set a 5 micron particle limit. That would be ok during the validation, but for routine monitoring I do not want to have a 5 micron limit for ISO Class 8 or ISO Class 8 at rest (Grade D).

A1: To conform to EU GMP, if a room is certified as 'EU GMP Grade D' then monitoring at both particle count sizes is the norm. The EU GMP Guide states, in relation to the limit, 'not assigned'. This doe snot mean 'not required' but that the user needs to define a limit. One approach could be to take an ISO Class

limit for the occupied state, although this is very high, whereas a limit set based on actual data is probably more meaningful.

A2: We currently have a limit of 20,000/m3 for 5 micron particle size in grade D at rest. I have not seen any out of spec results in the last 2 years that I have been reviewing monitoring results.

spread plating

Was wondering what is the maximum volume of liquid that can be spread onto TSA and SAB plates? Just out of curiosity :)

A1: I never spread more than 0.3 mL since greater volumes may not be absorbed by the agar.

A2: Depends on how dry the plates are. If you dry them out upside down on a iso 5 bench then you can probably do up to .5 ml however you will need more time with the hockey stick on the surface.

A3: You can spread 0.1 to 0.15 ml of liquid on to TSA and SBA plates because this quantity will gets dry on the plates quickly but if you spread more than this then drying will not be possible quickly and due to which you have to carry out spreading continuously on the same plate. This will allow agar particle to come out on the surface which is not acceptable.

A4: The answer to your question of what is the maximum volume of liquid that can be spread on a TSA/SAB plate depends on the size of the plates.
For a 100x15MM plate the maximum amount should be no more than 1.0ML. For a contact plate, use no more than 100 micro liters.

A5: It depends on the diameter of the plate. For a standard 9 cm plate, 0.1 mL will do. A 14 cm plate can have more (depending on thickness and Aw of plate) appr 1-2 mL.

A6: We would successfully place up to 0.2 mLs onto a 100 mm plate. We tried 0.5 mLs, however the colonies tend to run together once they grow. You can also increase your plate size to 150 mm, and we have placed up to 1 mL onto these - although 0.5 is probably ideal.

A7: It really just depends on how low you want your detection limit. We usually plate 0.1 ml on one plate. This allows for easy counting as well as quick drying time. However, we also commonly plate 0.5 ml on two plates to get a 1 ml calculation. I have also plated 0.33 ml across three plates in the past to represent 1ml.

Obviously, the more liquid you add, the longer it is going to take to dry. You are also more likely to have spreader colonies with the higher amounts. If you do plate a volume above 0.1 ml, I would make sure that you determine that your plates are dry before inverting them for incubation.

BIOASSAY READER

We are planning to initiate bioassay (Antibiotic assay) at our facility. I need information regarding performing bioassay and details of the bioassay zone reader instruments available in market.

A1: Giles Scientific (USA) sells the TRINITY V3 Microbiology System for automated antibiotic potency bioassays.

Visit http://www.biomic.com/TRINITY_V3/antibiotic_zone_reader_main_menu.html for more information.

A2: We purchased the Trinity V3 earlier this year and we love it. We were originally measuring zones of inhibition manually using a caliper which could take hours. The Trinity V3 is easy to use and it takes seconds to read the zones of plates.

A3: You want to introduce Bio Assay Reader in your lab. But Bio Assay Reader which are existing in market high price. Last years I raised a question regarding Bio assay reader like you. Few manufacturer sent me catalog and quotation. I am astonished for their price and I am totally dis-hearted. Why will be high price the Bio Assay Reader? What have in the reader ? Simple zone measuring device and a calculation sheet which we can prepare from MS excel. I do not want disappointed for establishment this. This is only for your information. Few types of Antibiotics such as Aminoglycosides, macrolides etc are performed Bio Assay. Maximum Assay are performed in HPLC or any others chemical method. Many companies have maximum ten types of antibiotics are performed Bio Assay. For small quantities of product, any company do not interest to invest huge money for Bio Assay. Manual reader such as Vernier Calipers that are now available digital form are used for measurement of zone of inhibition. Potency may be variable from man to man or laboratory to laboratory with this calipers. I think that it does not major factor.

TEST FOR SPECIFIED MO

I'd like to know your opinion and/or advice in this matter: when you're performing the test for specified microorganisms and dilution is the only way to neutralize the antimicrobial activity of the product, what is the maximum dilution volume that we should try in order to demonstrate that the microorganism couldn't be present in the sample?

Is there any reference bibliography?

A1: No there isn't any reference bibliography. The only thing you have is common sense and the statement in the harmonised pharmacopeia that if you can't pass the suitability test with your product (after 'suitable' amount of testing) you can conclude that contamination of that product is very unlikely (see Ph. Eur. 2.6.12 of USP <61>).

I have products which I have to dilute 1/1000 (1/10 for sample preparation and 1/100 for neutralizing antimicrobial activity) to pass the suitability test. If the specs for these products are 100 CFU/g this doesn't make sense, because when you do find a colony, it will be out of specs (and you can't count 0.01 colony). So our QA Manager decided that this proves that contamination of this product is very unlikely (Ph. Eur. 2.6.12 or USP <61>) and we don't do the dilution in routine testing. Our maximum dilution that we find sensible is 1/100 (1/10 for sample preparation and 1/10 for neutralizing antimicrobial activity). We also use this dilution for products that are very light or that swell in aqueous preparations (so 10 g won't fit in a 100 ml flask).

CCIT positive controls

I would like some feedback to help with an internal discussion. For those who are doing container closure integrity testing, either by dye ingress or by microbial ingress, how are you making your positive controls? What size holes or other disruption of the closure system are you using? These seem to be fairly undefined tests, and I understand from several sources that the agencies are starting to look closely at them.

A1: For our test method, we began by determining an LOD by using containers filled with HCL and varying amounts of the dye we use for CCI testing (0.1% methyl orange). The analysts that will be trained on the test procedure will analyze those vials (with a few vials with no dye mixed in) and document which are positive and which are negative. When the data is reviewed, we can determine the LOD for the test method. The LOD vial will be our positive control and a vial with no dye added will be the negative.

After each vial is tested (by both pressure and vacuum) the test article is simply placed on a white sheet of paper between the two controls for the analyst to make a comparison.

Documentation

I'm trying to benchmark whether firms require the original signed document from an outside laboratory that is performing GLP testing or whether the laboratory hold the original and sends a copy. In short, who maintains the originals? Both will be inspected. Thoughts?

A1: I assume you are asking for the testing release paperwork (final report or summary report)? Most labs I have dealt with have provided a signed final report to their customers. The raw data would stay at the lab, which is what I (or inspectors) would be auditing. Would like to hear what others have experienced.

A2: Contract testing lab keeps the original raw data and a copy of summary report; provides a copy of raw data and original summary report to client.

A3: We keep a copy on site and the original goes to the client. However, we dokeep the original raw data for a minimum of 7 years, unless longer is requested by the client.

A4: I have always received the original document and the outside laboratory (contract lab) stays with a copy. The data is reviewed by the contract lab but we do the final approval. These details are covered in the contract agreement.

Purified Water Test Sample Hold Times

Currently, our Water Testing SOP requires us to test our routine water samples within 24 hours after collection.

Our current practice is to test the water samples the same day that we collect them but there are times especially during system sanitization that we would collect water samples one day and test them the following day because we have to collect more than 40 samples (samples are stored in refrigerator).

USP <1231> Water for Pharmaceutical Purposes has been revised and the following guidance document states " it is best to test the samples as soon as possible after being collected. If it is not possible to test the sample within about 2 hours of collection, the sample should be held at refrigerated temperatures (2 to 8) for a maximum of about 12 hours to maintain the microbial attributes until analysis. In situations where even this is not possible (such as when using off-site contract laboratories), testing of these refrigerated samples should be performed within 48 hours after sample collection".

Since USP <1231> is a guidance document, should we revise our procedure to require us to test the water samples within 12 hours or can we keep the 24 hour allowance?

A1: Whatever you do you should have data to support your decision. Perform a qualification/validation of your hold time.

A2: You can validate the water sample testing after 12 hours and 24 hours of storage as per your existing procedure. Compare the results of 12 hours water samples tested with 24 hours water samples tested.

No significance change in comparative results of this study between 12 hours and 24 hours can support the long storage of samples i.e. what you follow presently. this would help you.

A3: I would sugget keeping the 24 hour allowance.

A4: What you need is to document that you are able to recover organisms within the 12, 24, or 48 hours- whichever you follow. Having the data to support your practice is the key.

A5: Most of your responses have given you the right advice. However the next 2 sentences from the <1231> text also tell you the same thing:

"In the delayed testing scenario [meaning that timeframe between 12hr and 48hr], the recovered microbial levels may not be the same as would have been recovered had the testing been performed shortly after sample collection. Therefore, studies should be performed to determine the existence and acceptability of potential microbial enumeration aberrations caused by protracted testing delays."

In other words, testing delays as long as 12 hr (with refrigerated samples) do not need to be specifically qualified, but going any longer does need to be qualified. The normal variability of this plate count procedure could mask a small systematic bias that could be caused by the hold time. Things you can do to reduce some of this variability include collecting a large sample that is well-mixed and subdivided into sample aliquots for immediate and delayed testing. You can also perform more replicate counts than usual, and if using a filtration test, test a larger volume to give a more reliable, higher colony counts (up to 300). You should also repeat the co-testing over several days of samples from a few selected poorer performing distribution outlets where the counts tend to be highest. Just do everything reasonable in this little study to remove unnecessary plate count variability and enhance the test's statistical reliability so that you could see a small difference in counts if it were to be present.

stability studies of BCG vaccine (freeze dried)

I want to prepare stability study protocol for BCG vaccine (freeze dried).

Real time study (2-8 degree)

I need guidelines related to the same and views regarding accelerated stability study.

Differential pressure limit

I would like to know about differential pressure limit requirement in clean room.
As per Schedual M 1.5 mmwc is required for clean environment so it is applicable to all class area? means i have 10,000 and 1,00,000 area in microbiology department so at what level i have to set my differential pressure?

CCIT - Extrapolating LOD for Different Methods

Dear [names redacted] and any others with interest in CCIT.

I'm aware of a recent PDA technical paper (Kirsch et al PDA Journal Vol 51 No 5) that describes a comparative study of Microbial Ingress vs Helium Leak Rates in relation to leak size and limit of detection in glass vials. The leaks of nominal size 0.1 - 10 micron were prepared by drilling 2mm holes in the wall of the glass vials and inserting micropipettes of various bore size into the hole and sealing in place with epoxy resin.

The threshold for Microbial Ingress was determined in this study to be 104.5 – 10-3 Std CC/second Helium Tracer Leakage equating to nominal leak dimensions of 0.4 – 2.0 microns. Below gaseous decay rates of 10-4.5 Std CC/second the rate of Microbial contamination was < 10%.
Critical leak rate Microbial Contamination was established at 10-5-10 -5.8 Std CC/second or in terms of leak dimension 0.2 – 0.3 micron.

As far as I'm aware the Helium Tracer method will provide sensitivities better than those of dye ingress, or pressure decay methods which can achieve LODs up to about 0.5 micron. However, the Helium tracer method is usually considered a lab reference method and not particularly suitable for routine QC use.
My own issue is trying to obviate the need for Microbial Ingress at all, because the package size is relatively large (250 – 1000 ml HDPE bottles of Neutraceutical product) and most contract laboratories (EU anyway) are more familiar doing vial and ampoule testing and are balking at the idea (clean up, logistics,

decontamination, biosafety issues). My hope is to try to establish a suitable rationale for testing of equivalence with a Pressure Decay or more likely Dye Ingress test.
So any suggestions are welcome. Here are some of my questions,

1: Container Closure Integrity Testing is mentioned and or recommended in various Official Guidelines and Documents and makes sense from a QA perspective (greater assurance) and I am also aware that there is a general movement towards the use of such methods to support if not supplant sterility testing at least as part of the stability requirements.

However my question is whether CCIT is actually a Defined Routine or Validation Requirement for;
A: Sterile Pharmaceutical Packaging or Medical Devices?
B: Sterile Neutraceuticals - Low Acid Packaged)?
C: Food (Low Acid Sterile)?
Any references to guidelines / regulations that explicitly state it as a requirement would be appreciated.

2: In literature from the late 90s early 2000s, Microbial Ingress was considered the gold standard against which all other methods were compared, is this still the case?

3: Can equivalence be extrapolated by use of existing reference studies like that mentioned at the start, that is if any are available for foil closures on HDPE bottles, rather than having to re-invent the wheel and initiating what would be quite a demanding Comparative Lab Based study?

3: Do any of the commercial Pressure Decay methods available achieve sensitivities greater than 10-6 sccs?

4: Is there a paper available that compares the Helium Leak Test with one or more common Dye Ingress Tests and shows any of these as having sensitivity of greater or equal to 10-6-10-7sccs.

New website for the advancement of rapid micro methods

I would like to make everyone aware of a new website for the advancement of Rapid Microbiological Methods. Rapidmicromethods.com (http://rapidmicromethods.com) provides a comprehensive introduction to rapid microbiological methods for the pharmaceutical and biopharmaceutical industries, validation strategies, regulatory perspectives, return on investment opportunities and technology reviews. Additionally, the site includes a discussion forum on topics related to rapid methods, a blog, a technology news update page, a calendar of events and an extensive reference page for rapid method publications and white papers that are either in print or available online.

The site provides a much-needed educational tool and scientific resource for anyone who is considering implementing a rapid microbiological method in their manufacturing or laboratory environment.

Parabens anitmicrobial effects

I am looking for information to understand how parabens (methylparaben, propylparaben, etc.) work to limit microbial growth. In particular, does the acid form of the paraben (i.e. para-hydroxybenzoic acid) form inside the microbe? Is it a product of metabolism of the paraben? Is para-hydroxybenzoic acid active against microbes? Also, does the FDA typically request for preservative related substance (i.e. para-hydroxybenzoic acid) in new drug products? Thanks in advance for any information you can provide me.

A1: The parabens disrupt the cell membrane and interferes with the transportation and energy generation. When cells are exposed, they will leak intracellular content but not lead to cell lysis or membrane damage. Cells will be able to recover when exposed to a preservative free media.
The pharmaceutical industry most commonly uses a mixture of esters: methyl and propyl ester.

A2: Actually parabens are banned from formulations and being replaced by safer products. And old products that uses them have to be reformulated at least in Europe.

A3: A great place to check for this and many other antimicrobials is Seymour Blocks' book, Disinfection, Sterilization, and Preservation . I'm not sure what the publication status is though. Other good sources are Dan Brannans' Cosmetic Microbiology: A Practical Handbook, or
Dave Steinbergs' Preservatives for Cosmetics

A4: Until your message below to the PMF, I was unaware that parabens had been banned for use in any type of product formulation. Please provide a reference that indicates that parabens can no longer be used in product formulations. According to the latest copy that I have of Cosmetic Directive 76/768/EEC Annex VI - List of preservatives that may be used in cosmetics, I still see that parabens still can be used in Europe (COLIPA Number P82, Reference # 12). In addition, the Cosmetic Ingredient Review had reviewed the safety of parabens in 2005 and had found them to be safe. If you have new information concerning the prohibition of these chemicals by a regulatory authority, I would like to review it. Otherwise, I'm unaware of it.

A5: This topic is very controversial and even if there is no directive to my knowledge that ban the paraben. A submission procedure with paraben will have to face difficulties since the topic has been discussed by European Food Safety Authority: EFSA Journal (2004)83 that can be viewed at the link.

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:52007DC0418:EN:NOT

A6: Dear Patrick,

Here are useful information about parabens.

Parabens-Statement of COLIPA : http://www.colipa.eu/news-a-events/statements/9-statement-chemicals-breast-cancer.html

Parabens-Statement of CTFA (PCPC) : http://www.personalcarecouncil.org/newsroom/20060901

Parabens-FDA : http://www.fda.gov/Cosmetics/ProductandIngredientSafety/SelectedCosmeticIngredients/ucm128042.htm

A7: I have to caution re. the data set behind this, Ruth. There really isn't good research into how the parabens work - membrane leakage of a moribund bacterial cell occurs with virtually all -antimicrobial treatment - physical or chemical. Hit the cell with a hammer and there's membrane leakage.

A8: Thanks for confirming there is no ban on parabens.

A9: Although parabens are legal, and are being used with no problems whatsoever in many cosmetic products, it is true that some European customers reject them. The claim that related parabens with cancer has not been forgotten, and manufacturers state on labels that their products are "paraben free" to be able to sell their products to these customers. Besides, the "natural cosmetic" fashion does not help, since certification bodies have banned the use of parabens and other "synthetic" preservatives.

bleach

Is anyone using a bleach solution to clean your cleanrooms? If so, what concentration are you using it at and what are your requirements in regards to contact time?

A1: The answer to your question is to use a 10% Bleach solution. The contact time should be no more than 15 minutes. I hope that you find this info helpful.

A2: We are using 500 ppm bleach solution acidified down to approx. pH 7 with 5% acetic acid. Contact time is 10 minutes. We use it only sparingly (e.g. once per quarter) or as needed to control spore-forming organisms. It is also used once when coming out of cleanroom shutdown or re-qual activities.

A3: Hi Jenna,
Bleach or sodium hypochlorite is still used for disinfecting non-product contact surfaces in cleanrooms and it is effective (though has a down side in that it is corrosive even on stainless steel).

It has been suggested that it is mixed with acid. I would be very cautious mixing bleach with any acid. Bleach is stabilised at alkaline pH (becomes more stable but slightly less effective) NaOCL dissociates in water to form HOCL hypochlorous acid . As the pH moves toward neutral and then to acid there is a shift in dissociation of the molecule eventually producing free chlorine gas which is highly toxic and of course very dangerous.

Use stabilised bleach by all means it can be purchased at in-use concentrations of about 1000ppm. and read the H&S data supplied with it. This will be sporicidal but you will have to define your own "wet" contact time. You will be lucky to achieve 5-8 minutes in a cleanroom with high air flow rates, so if your organisms (I presume spores) are tough you may have to apply more than once.

I would also recommend a water rinse afterwards to remove residue and minimise corrosion.

A4: For general disinfection (not cleanrooms) we used 10% Clorox solution (commercially available) with a 10 minute contact time. Works well with most organisms, however may not be completely sporicidal. You would need to validate this.

A5: We use Steris disinfectants for cleaning, "Environ Vesphene st" at a 1:128 dilution with sterile water for vegetative cells and sporklenz ready to use for sporicidal purposes. We have been rotating Vesphene with Lph st on a bi-weekly basis but I realise this practice is now redundant so if I would not recommend introducing such a rotation if you are setting up a cleaning regime.

A6: You may be interested in a study done in response to the anthrax scare several years ago. The use of acidified bleach was studied and performed exceeding well in killing microorganisms. The key was to acidify the bleach, and at a level of 2500 ppm it was quite effective.

See http://www.microchemlab.net/biodefense.htm

A7: Acidified? I'd be more specific here so as to avoid some health risks. It's well known that hypochlorite works best at slight acidification - ~ 6-7 but too much lower and ones risks exposure to chlorine gas.

A8: Everybody replied the correct. Chlorine solution may be used in clean room but main problem is corrosion activity of chlorine. After use of few months of chlorine epoxy paint turned fed colour. Residual content of chlorine effect the outer surface of machine. For that reason, after use of chlorine, 70 percent of IPA must be used to remove the residual content of chlorine. It can be helpful to prevent contamination in Clean room.

A9: please have attention to the following test, written in chapter <797> FAQ of the USP:

40. Can bleach be used as appropriate cleaning agent and can diluted bleach be used exclusively as a disinfectant?

Bleach can be effective as a disinfectant but is inactivated by proteins. Bleach is not appropriate for disinfecting critical sites. Bleach is appropriate if followed by sterile 70% IPA wipe (see question #34) Consideration for the selection of cleaning agents should be given to the effect on surfaces and potential respiratory, skin, and eye irritation to the operator.

B. cepacia in cosmetic product

In the recent days we found B cepacia in a Absence/Presence test of P. aeruginosa in a hair treatment. In my country the identification of this microorganism is not required, but We prefer to reject the batch of product, because of the documentation about the risk in people with chronic diseases, children and older people. I know it´s a hair product but still, it´s so risky. And now we need to investigate the root of this contamination in order to eliminate it. What do you think about the rejection of a cosmetic product like this, when B. cepacia is found, and what do you think about why is not a requirement in routine analysis?

A1: It is my opinion that you had made the correct decision from your risk assessment to reject this product formulation that was found to be contaminated with Burkholderia cepacia. Besides of the hazards that you had identified in your email below to users of the product, the presence of B. cepacia in a finished product can also have a detrimental effect on the formulation (e.g. loss of viscosity, color changes, malodors, etc). I suspect that the root cause of this Burkholderia cepacia contamination was due to either the use of contaminated water to make the batch (e.g. water system) or improper cleaning /sanitization of the manufacturing equipment. It is very common for some companies to use tap water to conduct a portion of the cleaning process for manufacturing equipment. The tap water may have been contaminated B. cepacia. I have found that the reason as to why companies uses tap water as part of the cleaning process is because it is cheaper to use than deionized or distilled water in the removal of product from equipment surfaces. As far a microbial test specification, each company has to make a decision as which microbial species are acceptable or not acceptable to be present in your products. You will need to have a justification as to why you would allow certain species to be present in your product formulations.

A2: We found B. cepacia in a hair gel we were trying to bring into production about two years ago. As a gram negative organism, we rejected the batch due to presence of "objectionable organisms" which may be why it is not specified like Ps. aeruginosa.

As a hair care product it is unlikely to be inhaled (most common route of infection) but there have been cases documented of skin and eye infections with B. cepacia. We saw evidence that B. cepacia was actually metabolizing some of the parabens in the preservative system, leading to further concern.

B. cepacia is endemic to many parts of the world and can be found in water and soil. It used to be Pseudomonas cepacia until the pseudomonads were divided up into multiple genera, which may account for the method of identification in your case. We first found growth on our standard SabDex plates.

A3: The detection of a Gram negative in Cosmetic product should be investigated to determine the root cause and prevent the rejection of additional batches. I am in alignment with your decision of withholding product disposition but this decision should be part of your investigation process. I wouldn't have made a product disposition decision without having done the investigation. Why is B cepacia a nightmare? It has its ways of adaptation to preservative systems and can be difficult to get rid from systems. You should pay close attention to the following:

1. Water system- Remember that Burkholderia cepacia is a waterborne microorganism. Investigate the water results the date the product was manufactured and when the equipment was cleaned and sanitized. Also pay close attention to how water is added during manufacturing and product transfer lines.

2. Cleaning and sanitization (c&s) practices- Inadequate c@s practices and improper equipment draining can lead to the formation of biofilms. Check for deviations from the acceptance criteria of temperature, exposure time, frequencies, etc during these sanitizations. If water is not completely drained from a system it will eventually lead to bigger problems as well as if the equipment does not reach temperature or time during a sanitization process. If a system is not adequately cleaned microbes can be protected under these product residues. Keep an eye on these problem areas.

3. Do you have an ongoing method to determine your sanitization effectiveness such as equipment monitoring? If so, have you had any detection?

4. Were your detections in Total Plate count and Enrichment? Were your detections in all your product samples? The answer to these questions can help you understand the whereabouts of your contamination.

5. Raw Materials- Verify testing results.

A4: Many years ago (70's) we found the same organism in a shampoo product. The batch was rejected. The source of the contamination I believe was biofilm in the water system.

A5: Contamination with excessive populations of B. cepacia is the most common microbiological cause of cosmetic recalls.

routine non-viable particulate environmental monitoring

I'm curious to know what the industry standard is for the number of sampling sites required for routine particulate counting. ISO 14644-1 section B.4.1 defines an equation for the number of sites required for cleanroom qualifications, but I'm hoping most are using a lesser amount of sampling points for routine environmental monitoring.

Homogenisation equipment in microbiology

I assume that most of you are using some kind of homogenisation equipment (incubator shakers, stomachers etc.) while preparing sample solutions for microbiological examination.

Have you ever considered what effect that kind of homogenisation has on microbial contamination and tried to justify the use of it? Have you ever been asked to do so by relevant inspection bodies?

I use a type of an incubator shaker on a daily basis, and I tried to shake some inoculated solutions (standard pharmacopoeial test microorganisms) - plated them before and after the shaker (30 minutes at 350 rpm, 40°C). Only B. subtilis gave me a hard time - apparently shaking kills it - the numbers after the shaking were significantly lower (more than 50% lower).

What were your results?
Do you have some references that deal with the effect of mechanical force on microorganisms in general?

Origin of Plate Sizes

An interesting question posted earlier in the week on the PharmSciTech list is reproduced below. I have no idea as to the answer - can anyone help?

"Can you please explain the rationale behind selection of size of plates i.e. how size of plate is selected for monitoring of environment and how 90 mm or 55 mm size has been originated for use."

filtering adjuvant

Does anyone know of a way to solubilize alhydrogel adjuvant (alum or aluminum hydroxide) that does not interfere with the microbiology? I'm looking for a way to make this easier to filter. (Alhydrogel is found in some vaccine formulations.).

A1: Why you want to solubilize alhydrogel...please be clear what you are going to perform. Is this need of any test or what?

A2: It is in the context of filtering for a sterility test.

A3: If you have already validated your process of sterility with respect to the vaccine containing alhydrogel then you can take reference from it or what else you can do is give multiple rinses of the sterile diluent (3 times) which enables the product to pass easily. This type of problem occur for adjuvant/formulated vaccine. Even you can go for direct inoculation method.

A4: This is in the development phase. We do not have a validated process. What sterile diluent enables the product to filter easily?

Staphylococcus haemolyticus

I work for a pharmaceutical company that manufactures prescription transdermal patches. We test all of our raw materials and finished product lots for microbiological growth through a contract laboratory. Some of our packaging film came back with bacterial growth on the MSA and TSA enrichment streak plates which have been identified as Staphylococcus haemolyticus. This material is used to produce the pouch in which our patch is placed. According to the test report the number of colonies found is <10cfu/gm. Is this something we should be concerned about?

A1: Based on the data provided, your isolate must have been recovered from Enrichment test and not from TPC. The fact that S haemolyticus was found in the packaging film is indicative of human interaction with the packaging material as judged by its human source. How did it get introduced to your packaging film? I would assume that it was either introduced during the manufacturing process or during the laboratory testing of the sample. Have you had other recoveries of similar flora in previous lots of this product? Were your test results on different lot samples of the packaging material? How about the results of your finished product? The answers to these questions will surely help in understanding the possible source. Since the survival of S. haemolyticus in such a packaging material is questionable due to the environmental conditions (habitat) for subsistence, I would suggest further investigating to draw conclusions.

Although I assume that you are within finished product specifications, I would treat these packaging film results as an alert. The investigational process will help you understand the source and the microbiological risks to the product.

These detections can start with S. haemolyticus and be followed by S. aureus. Then you will have a problem that could have been prevented during these warning signs.

A2: Other lots of the packaging film have shown everything from no growth, to growth of Bacillus subtilis/ amyloliquefaciens/atrophaeus, Staphylococcus epidermidis, and Bacillus pumilus, not all at the same time of course. The highest count was 40 cfu/gm.

The sample with the Staphylococcus haemolyticus result came from 1 roll out of the lot of packaging film.

Our finished product has come back with growth a few times. The identification of the growths on our finished product have been Bacillus subtilis/amyloliquefaciens/atrophaeus, Staphylococcus intermedius, Staphylococcus sciuri, Pseudomonas oryzihabitans, and Pantoea spp.

A3: Staphylococci species are typical human-borne isolates and represent a large % of microorganisms isolated from environmental samples in pharmaceutical facilities. The likely source of this type of contamination is inadequate aseptic technique and gowning practices, and or poor clean room behaviour. As part of your investigation, you may need to look at environmental micro records to see if any out of trend results, environmental swabs results of clean equipments ect...

As these organisms come from human, you need to look at manufacturing practices, aseptic sampling techniques by sampler etc.

Lastly, you should enquire about the procedure that took place in testing your sample by the contract lab(it is very easy for laboratory analysts to contaminate samples during testing)-did the contract lab test the sample in the laminar flow? did the analyst(s) involved gown up (gloves, hair nets, face masks etc.). The final laboratory investigation results may allow you to retest.

As coagulase negative staphylococci are predominant members of the normal human skin flora and are generally considered non-virulent, these organisms are also being increasingly recognized as opportunistic pathogens. Are these patches applied to intact skin? As gram +cocci tends to withstand desiccation better than Gram -ve, they may survive longer in your product also.

A4: Thanks for sharing the typical flora reported from your packaging film and finished product. In trying to answer how the S. haemolyticus got introduced, I still think a closer look at the testing process of the contract lab is a must and an audit will provide you with lots of information and risk areas. Do not forget to check their testing controls and environmental monitoring results. If not available, request it. Take a close look at your sampling and sample transportation; these could still be contributors to the packaging film findings.

After reviewing the bioburden of your finished product, I see three main groups:

1.Typical Environmental Flora (B. subtilis, P. agglomerans)
2. Human skin commensals (S. intermedius and S. sciuri)
3. Waterborne microbes (P. oryzihabitans).

These are not considered objectionable organisms for most non-sterile finished products. Since I am not familiar with your product and have very little known facts, I am not able to make any final conclusions but assume that this bioburden is not objectionable and typical of an environmental and water flora at a pharmaceutical facility with the exceptions of your Staphylococcus not typically seen in the finished product that I have worked.

Adequate controls and preventive measures ought to be in place to reduce and eliminate their incidences. Some examples are provided:

1. Protective Garment- this prevents human commensals.
2. Effective sanitization, equipment draining and drying- this prevents waterborne.
3. Storage- Prevents Airborne (dust) from reaching into the equipment.
4. Equipment design- Sanitary design.

The list goes on and on…

USP 62

If you observe growth less than 18 hours is this acceptable for suitability testing?

A1: The intent of the suitability test in USP<62> is to demonstrate that the conditions of your testing will allow recovery of the specified organisms. You did not state if what you tested had a monograph detailing the required test. If you refer to section 6.30, "Alternative and Harmonized Methods and Procedures" in the USP General Notices, you should find your answer.

In any case, wouldn't you be concerned with your test meeting the GMP requirement of "absence of objectionable microorganisms"? Will the less than 18 hour incubation period allow you to recover your objectionable microorganisms?

A2: The purpose of suitability testing is to prove you can grow the micro-organisms within the shortest period of time. What test is this result for?

A3: Yes, the characteristic growth before 18hrs, proves that this media is of fine quality,

A4: While performing suitability test pseudomonas who has longer generation time than E.coli are difficult to recover with less incubation in the broths with neutralisers. Pseudomonas may grow in simple broth faster, but in certain product/ broth dilutions it has taken longer to grow.

A5: In general *for validation and suitability test* for MLT and AET, recovery should be in lesser(Minimum) incubation period (proves the method is suitable for the particular product) but for testing especially absence of growth, one has to wait maximum incubation period

Reference: USP1227

Objectionable organisms in non-sterile oral dosage

My question relates to the process in the determination of if an isolated organism(s) is objectionable or not objectionable. As this question relates to non-sterile oral dosage (tablets and capsules) and the assumption is that it has meet all pharmacopeias methods (TAMC, TYMC, E.coli, Salmonella spp. ect...0).

How would you determine if an organism(s) detected is objectionable?

As per [name redacted] news letters and the Pharmacopeias, the risk analysis should incorporate a minimum of four separate analyses:

1) Absolute numbers of organism(s) detected.

In this case we are dealing with TVAC <1000cfg/g as it has meet specs.

2)Microorganism's characteristics

Some of the common organisms may be Enterobacteriaceae (Enterobacter spp, Citrobacter spp, Cronobacter spp, Escherichia spp ect), Gram+ bacillus (B.cereus) and Gram +cocci (Staphylococci spp)........This information is available from scientific literatures.

As the tablets and capsules have low water activity (<0.6) so we dont expect proliferation in numbers.

3)Product Characteristics

low water activity tablets and capsules.

4)Patient population.

As [name redacted] have previously mention in his Newsletter, the manufacturer cannot control and should not be held accountable for abuse of a product or off label use of the product by physicians. Many tablets may be used by the physician for infants and young children. Many immunocompromised patients with underlying diseases and on immunosupressive medications may be highly susceptible to infection.

Please advise and comment on the hypothetical Scenario below (please state whether you would reject or release product for sale.

Scenario 1

A tablet X meets all pharmacopoeial micro tests. However, Cronobacter spp (previously Enterobacter sakazakii) was detected from the Salmonella test(/10g), the GN Bile Tolerant test was not detected/g. TAMC was 50cfu/g and was not identified to species level.

Note: some Cronobacter spp may be inhibited by crystal violet or other components in the test.

Scenario 2

A capsule Y meets all pharmacopoeial micro tests. However Escherichia spp (not E.coli) was detected from the Salmonella test. The E.coli test result was not detected/gram. TAMC was <10cfu/g.

All identifications performed to genetics level and all investigations performed to rule out laboratory and or sampling contaminations.

A1: •An objectionable microorganism can be defined as: 1) an organism that can proliferate in a product adversely affecting the physical and therapeutic attributes of that pharmaceutical product, and 2) an organism that due to its numbers in the product and pathogenicity can cause infection in the patient in the route of administration when treated with that pharmaceutical product.

A2: I face the exact same scenarios every week, and in fact deal with those same two genus/species.

For Cronobacter sakazakii, the literature offers that the incidence of adverse reactions caused by this species are very rare, and restricted to neonates and infants under 6 months. This is clearly because the main source of incidents are related to mishandling of infant formula, which has been held at warm temps too long, allowing low levels to proliferate to an infective dose. I take a broader, safer view, and we only permit a low level of detection to be used if the final products have label warning ("not for use by people under 15 years") or designed application for healthy adults; we take this even further by formally risk assessing, with signed statements from the QAM, Ops Manager and Microbiologist, addressing the risk of that healthy adult giving their neonate a taste; of course we can't stop that happening, but we cover ourselves by actually calculating the risk of it happening, and then formally accepting that level of risk (or rejecting, if the risk is too high; a business decision). One significant aspect of your risk asses for Sakazakii is that you are dealing with a tablet; how many neonates do you know that could swallow a tablet? Not many I think! Is that tablet likely to be dissolved in warm water, kept warm for several hours and then given to an infant? Doubtful!

For Escherichia spp., (but not E. coli), note that E. coli is an indicator of faecal contamination, and one can assume that other, nasty bugs will be present if E. coli is found. This assumption is not true for other Escherichia spp..

In both your examples, you isolated from the 10 gm Sal test, and not from the 1 gm samples applied to other enteric tests, which is common situation, and suggests low level contamination. I seriously doubt that any data you present would cause stability or efficacy or degradation issues, but that's only based on your low Aw tablet/capsule info...

In both cases, I would, have, and will continue to release my products with these type of isolations, but each accompanied with a formal Risk Assessment, in accordance with policy, and approved SOP, and recognised training from an external Risk trainer; all our senior Risk signatories have qualified for Risk Assess at a degree unit level. The key point though is that my standpoint only applies to my products, and I can't really offer a firm decision for you and yours.

In further defense of this standpoint, we still acknowledge the inherent poor quality of the products under test and continually strive to investigate and improve so that these "undesirable", but not "Objectionable" isolates be reduced.

As microbiologists all we can do is present the data and actively, professionally and credibly participate in the process that the business takes to approve or reject; we can prosecute to the highest

degree based on our knowledge of pathogenicity and infective dose, and if the company overrides that decision then you are stuck between the proverbial rock and hard place. The final straw, if you really honestly believe that the company is endangering customers over your head, is to blow the whistle. In my state/country there are 6 species that I am required to notify government of if I find them; of course I'll let my bosses know I'm going to do it, but I won't break the law just to protect them, and conversely, if they attempted to prevent me from doing so, they'd be in big trouble!

A3: Your product specification should already list the most typical pathogens that are unacceptable. As per USP pharmacopeia requirements, these are E. coli (USP <1111>). Other organism typically considered objectionable is Salmonella spp. Other clinically relevant Enterobacteriaceae can also be considered objectionable but are not typically listed and require a risk assessment.

The four items from Scott are typically part of this risk assessment and will require to be evaluated during the investigational process.

As part of the risk to the product process, I would also assess the following:

1 The physiological and survival strategies of the isolate. Some isolates such as Enterobacter sakasakii have a high tolerance to desiccation and thermo tolerant.

2. The potential of the microorganisms to spoil product and its threat to the patient (medical assessment).

In your hypothetical scenarios I would do as follows:

Scenario 1:
In order to make an accurate risk assessment it is critical to determine the species that was recovered. Enterobacters are members of the Enterobacteriaceae family and can either be associated or not associated with human infections. Its presence in product is indicative of its survival through the manufacturing process and its presence in either the equipment (biofilm) or associated to a raw material. I would see its presence as of high risk to the product and the customer, I would not release product.

Scenario 2:
Escherichia species are not commonly associated with human infections contrary to Escherichia coli. I would further test the isolate (TSI and LIA reactions, etc.) to try to screen the microbe. Product could be released but it should be extensively substantiated.

A4: I already discussed the issue of microbial bioburden on solid dosage forms on an article that I published on PharmTech in 2002. The title of the article is "Microbial bioburden on solid dosage forms" and it still applies today. You can find it here: http://pharmtech.findpharma.com/pharmtech/Microbial-Bioburden-on-Oral-Solid-Dosage-Forms/ArticleStandard/Article/detail/9672.

Endotoxin removal from WFI system

Few times Gram negative bacterial may be present in WFI system. Specially after holiday it may be occurred in water pipe line or after collection of water for processing, rest part of water may be responsible for producing of endotoxin. My question, how many time need for producing of endotoxin from Gram Negative Bacteria ? How many cell can be produced 1 EU ? If present endotoxin in WFI line what action to be taken to remove this?

GPT

How should we do GPT ,I mean when we receive a media (Powder or ready) we do GPT only once and we do the controls for each sterilized media. if this is correct anybody has an idea where can I find it in Canadian GMP or USP.

Some people believe that we have to do GPT each time we sterilize a media, do you think this is correct & why?

A1: Harmonised Ph. Eur. And USP state that every batch of ready prepared medium should be tested. That means every time you receive a batch and technically every time you prepare a batch. We test every batch that comes from our supplier and once a year we test the dehydrated media for growth promotion and every time we make a batch we have a negative control. But we don't test every batch we make for growth promotion.

A2: There are different opinions of people about this topic. Recently when we are also preparing Standard Operating Procedure for same we found in some guidelines where it has been mentioned to perform GPT with every new batch.It is little bit confusable that 'new batch' of dehydrated media or 'new batch' regarding daily media preparation, or 'new batch' regarding individual dehydrated media container.
But my personal opinion is we have to perform GPT with daily sterilized media.It will define nutritional quality of your daily sterilized media more precisely.

A3: as per usp, one should do gpt of each autoclaved lot of media or each lot of media that you receive as ready prepared,

A4: Your 2nd statement is correct ie we have to do GPT for each autoclaved lot
of media as per USP/EP/JP.

Reason: Your autoclave temperature and pressure should not affect your media quality
(Though it is validated).
Each time preparation media we may use different glassware
which was used for selective and differential media (improper cleaning but
invisible)
Interference of cleaning agent used for washing of
glasswares
Preparation method (Qty variation, water variation...)
Storage period (Duration between receiving new lot and final utilization of
media for testing)
Should not affect the growth promoting ability of the media.

A5: How a bout checking the pH and appearance of media after sterilization or just streaking organisms on the media?

A6: I put my question this way, could we do the controls + & - on each prepared batch (autoclaved one) instead of GPT and do GPT only once on received media (Dehydrated or Ready) if your answer is yes can I prove this as conform to USP/EP....Does the controls + & - are acceptable.

A7: As per USP-33 General chapter -62 it is clear mention that you have to check growth promotion, inhibitory properties with suitability of media for every new preparation of media lot or batch, so nicely refer this chapter.

A8: I want to share my knowledge with you regarding Growth Promotion Test of Media. I agree with all. Actually it is very difficult to carry out GPT for each lot of prepared media. Dehydrated media after receiving from supplier it should be done for each lot of media. I think main factor is the concentration of inoculum which is 10 to 100 cfu those are unstable during preservation. GPT must be performed for different standard microorganism as per USP with the above concentration. Everybody will say that it should be done but please think, you have to prepare 10 to 100 cfu everyday for GPT that is not easy.

If I am wrong please tell me what is the right way ?

A9: [name redacted], I agree with you but what strains will you use on a daily basis, I mean are you going to prepare less than 100 cfu/ml on a daily basis.

[name redacted], I agree with you ,when you consider the volume of the work it is not going to be easy to do GPT on all sterilized lots with < 100 cfu/ml

A10: [name redacted], I totally agree and have had the same difficulty with maintenance. However, using a commercial prep of the 10 to 100 cfu for each organism in easy to use and store configurations is extremely easy. You could even use a certified/quantified suspension with a population for example of Log 4 per ml and with simple dilution, have your 10 to 100 cfu's with the remainder of the suspension vial to use for possibly 100 or more future GPT.

A11: I run the quality department for a contract micro laboratory. Our suppliers of dehydrated media are already running a selectivity test. So, our quality system allows us to utilize the supplier's certificate of analysis for the selectivity testing. This requires more control of our suppliers which might require an on site audit. This is the approach the laboratory takes for the approval of purchased media. Then based on the purchase history of the media, we perform periodic testing to confirm the supplier's certificate of analysis.

Every batch of prepared media made is tested with a positive control for growth promotion to confirm the media allows growth as well as a negative control to confirm the media was sterilized.

Being a contract laboratory, this is feasible because the amount of media produced. We keep live controls at all times. Your situation might be different and the cost of quality might be more than your company wants to maintain. Knowing your media is capable of accurate performance is vital, if this information is not able to be maintained, consider contracting the work out to a company that can maintain such quality.

A12: I agree with you in each word, but what do you say about this idea, to prepare cultures with 10 - 100 cfu per ml. Then distribute them in mini vials and keep them in very low temperature - 20 as are kept in biotechnology processes. We can use them when need them.

A13: I just wanted to add some clarification and answer your question on what should be the right way, I hope this helps.

First, we must understand why is growth promotion required? It is to have assurance that our sterilized media is capable of sustaining microbial growth when these challenges are at low numbers.

So what factors could affect the microbial growth of a media?

1. Lack of uniformity in the Dehydrated Media- I basically refer to the adequate combination of ingredients in the powder (i.e. proteins, sugars, etc.)
2. Inadequate storage conditions of the dehydrated media- Some media are hygroscopic.
3. Incorrect Media Preparation- Incorrect weighing, water addition etc.
4. Overheated media- exposure of the media to excessive heat leads to denaturalization of proteins.
5. Poorly cleaned glassware

So if any of these factors takes place during the media preparation, the quality of the media would be compromised together with its capability to support microbial growth. Then, isn't this one of the reasons why we do growth promotion to media? We want to make sure that the media is capable of supporting microbial growth and of low microbial level detections in tested product.

Now the question is a matter of frequency. How often should Growth Promotion Test be performed?

The USP Guidelines in <1117> Microbiological Best laboratory Practices provides guidelines on media testing frequencies:

1. Quality control tests should be performed on all prepared media.
2. Tests ought to be performed routinely on in-house prepared media (i.e. pH, growth promotion).

3. If the in-house media preparation and sterilization are VALIDATED, then the growth promotion test may be limited to each incoming lot of dehydrated media. (Always verify the compendial method for further requirements).
4. If the in-house media preparation and sterilization are NOT VALIDATED, then every batch (lot) of media would be subjected to growth-promotion testing.

So the only way to reduce the amount of media lots tested is by validation of your process.

Testing with low microbial numbers of less than 100cfu is a most. Actually, most manufactured products usually have a low microbial content. Our media wants to target the recovery of such low levels to prevent the release of such product if it happened to be out of product specifications.

A14: Really is better to carry out the GPT in each lot of prepared media in the laboratory, because there are some things that could be affect the quality of culture media during the preparation, for example, if you culture media is exposed to heat during long time, the capacity of recover microorganism can decrease or the morpholgy can change (normally, the colonies are more small).

May be you can preprogram the GPT for 1 day in the week, so you can make the dilutions of the microorganism once in the week.

A15: Based on the factors that you enumerated which may affect growth promotion or/ and the quality of the media, to be on the safe spot, I think every lot of media sterilized should be tested for: pH before and after sterilization and Growth promotion test. We all know some time USP is very hard to read or to understand the wording. As a scientific we have to use our commun sense to interpret and understand what is the points of using USP as guide lines.

A16: We do GPT of inoculum for bacteria on TSA and inoculum for Y&M on SDA spread plates. We aim to get counts of less than 100cfu but more than 25cfu (lower countable range). We still accept counts of lower than 25cfu inoculum only for broth type media. For selective media plates where a % recovery is calculated, we use inoculum in the range of 25 cfu to less than 100cfu.

Can anyone please tell me if <25 cfu is still countable (will it be accurate at this lower end)?

Does anybody do a shelf life validation of the powdered media (especially if the powder has a shelf life of a few years, it tends to be used up before this time). If not, then how do we know that the powdered media will still be good to use at end of shelf life?

A17: Yes it is difficult. but one has to follow the Pharmacopoeia and we can plan GPT by the preparation of media weekly once/ twice in a bulk manner and by doing some validation(one time activity). media storage validation and CFU vs turbidity unit measurement validation.

For CFU vs turbidity unit measurement validation, one can use *McFarland Turbidity Standards *(densitometric method).

Here one has to validate the CFU vs turbidity unit measurements before using this standard. After the validation, the bacterial and yeast suspension can be used immediately and by practice, one can easily select the dilution having 10- 100 cfu. This can be cross verified by using already GPT passed media.

But the bacterial and yeast slants should be ready on daily basis.

This method is very useful for AET as per EP which states that the bacterial suspensions should be used immediately.

Biomerix and BD diagnostics supplying the Opalescence/turbidity standard. refer below link

http://www.keyscientific.com/McFarland%20Standards.pdf http://www.microbiol.org/white.papers/WP.OD.htm

A18: Good example question on the need to do GPT on each batch of media. This is why we do so, to see if it is still 'good to use'. The daily testing can be very easy and not difficult. If one has a certified population for a 10 ml suspension of 6633 spores at a population of Log 4 per ml. It is quite easy to dilute down to 10 to 100 cfu's or extract 0.01ml and add to dilution blank to get 10 to 100 and test. Suspension is validated for at least a 1 yr. shelf life so you still have 9.9 ml of the suspension left to do another 99 GPT from the vial. With the suspension vial cost, this is about a cost of $1.00 per test with this organism. Then go to the other organisms. There are several such available predetermined commercial population kits that are already at 10 to 100 cfu's available for all GPT organisms. The cost of the kits is far less than what would be your time and effort cost to maintain a culture and verify viable population that still exists in your culture.

A19: Our environmental monitoring media is tested for GPT by the manufacturer, using these microorganisms: C. albicans, S. typhimurium, A. brasiliensis, S. aureus, B. subtilis, E. coli and P. aeruginosa. We perform GPT on the media, upon receipt and every 90 days until expiration, utilizing B. subtilis, A. brasiliensis and C. albicans only. Are we meeting all the necessary compendial requirements for growth promotion testing for environmental monitoring culture media? (We do not perform sterility testing or microbial limits tests at this time.)

A20: In USP <61> its written that TEST EACH BATCH OF READY PREPARED MEDIUM AND EACH BATCH OF MEDIUM PREPARED EITHER FROM DEHYDRATED MEDIUM OR FROM THE INGREDIENTS.

My question is suppose i prepared SCDA (SOYABEAN CASEIN DIGEST AGAR) 3 times in same day for different purpose, than I have to do of SCDA three times or one time.

A21: You have to do GPT on each autoclaved batch, means 3 times in your case.

A22: Yes, for each new prepared lot and for each new autoclaved lot.

A23: GPT is for individual Batch when you purchase any media it have batch no. you only check once as per media characteristic & record it in GPT Log Book or protocol.

If you are preparing any media of same batch multiple times you just perform its blank for positive or negative growth. Treat this media as sample incubate in the same manner Its for your media sterility check its proves proper autoclaving of media . Record the growth observation in media preparation record.

A24: I think you should test each prepared lot of SCDA, because you will autoclave and prepare it so it ought to be checked for GPT as well as for sterility.

In case of readily available SCDA plates you shall test GPT at least once for each lot you purchase, considering the expiry date, hold temperature and time and also the retest frequency of media.

Sometimes in case of the same lot number of the media you have to test GPT for more than one time if you hold the media for longer period of time within expiry date.

A24: GPT needs to be performed on media from each autoclave cycle, showing that each cycle "produced proper media".

A25: "I prepare SCDA three times in same day for different purposes", regardless of purpose, one needs to test each 'newly' made batch. If all were made at the same time, all in the chamber at the same time, then testing is reduced. With GPT one is testing ability to promote growth for media quality and also for media sterility... three separate media loads require three separate tests, one for each cycle run. Cycle 2 and 3 may have encountered problems with steam quality, placement, additional unexpected time, etc. that did not happen in the first cycle you tested and passed.

A26: If you are giving different batch no. of all three SCDA media then you have to do gpt for all three batches of SCDA.

A27: The lot terminology different companies are using many way. Make it one think clear I have prepared SCDA in 250ml, 500 ml,1000 ml, and autoclaved in same (one) cycle. Should I have to do GPT for all 3 different containers? if your answer is yes, why?

A28: No, provided your autoclave cycle is validated to process all three volumes together.

A29: We use the minor volume because it is the worst case, because that containers reach the sterilization temperature before the others, so the exposure time is the highest.

A30: You need to do GPT only for one cycle.The number of containers can vary.One cycle of autoclaved media should be tested for GPT.

microbiology reference

I'm looking for the best microbiology reference (Book) which could be useful in pharmaceutical, cosmetic, natural product field.

A1: My recommendation is Pharmaceutical Microbiology, edited now by Denyer, Hodges and Gorman (originally Hugo and Russell) published by Blackwell Science Ltd.

A2: Not aware of a single ref that effectively covers all these. For cosmetics, I'd recommend a text that I edited - Handbook of Cosmetic Microbiology 2nd ed. Chapters by some experts, like Dr. Sutton, are focused on the practical.

incubator validation

I have a very small incubator that I would like to put in service just for incubating Quanticult vials for growth promotion. This incubator is set up to be monitored by a thermometer. Ideally I would prefer not to put a chart recorder or continuous RTD monitor in this incubator as it is so small and only used for 15 minutes periodically. Does anyone know if I could just have the analyst write down the temperature when the Quanticults are placed in the incubator and when they are removed if this would meet GMP compliance?

A1: As per experience, I have used a small incubator for such purposes and the analysts would record the temperature reading when placing and removing the Quanticults during rehydration (dissolving of the pellet). The purpose of this step is rehydration, if not effective, it will be reflected in the recovery. No concerns from the auditors were made known.

Anyhow, before this incubator was put in use, we did its Installation and Operational Qualification (IQ/OQ). The operational qualification gave us the guarantee that the equipment was capable of functioning at a wide temperature range and was maintained and constant through time. We used a continuous RTD monitor during these qualifications.

A2: It would meet GMP requirements. Because you are using this incubator for the incubation of microorganisms used in GPT, I would take more cautious approach by verifying:
1. IOQ and temperature mapping completed.
2. Door opening/temperature recovery studies (critical because you are incubating Quanticults for only 15 minutes). Temperature loss/recovery may not be a issue considering you have small incubator.
3. Possibility of using high/low thermometer.

Vaccine Production

Regarding Vaccine production, is there any guideline or regulatory problem if we produce Bacterial Vaccine & Viral Vaccine in the same production line (Pre-formulation, Formulation & Filling) with highly consideration for cleaning process.

Non-sterile TSB for media fills?

I am looking for some expertise with respect to media fills.

Is it a requirement to use non-sterile media for performing aseptic media fills? For aseptic sterile filtered processing, and with microbial retention validation of the filters, is there still a regulatory requirement to use non-sterile media? I am unable to find this as a requirement anywhere, but I wonder if this might be industry practice.

A1: If the process being validated includes a sterilizing filtration as part of the fill operation, it's permissible, but NOT required by anyone I've ever heard of, to use nonsterile TSB, but you should also know HOW nonsterile it is. I wouldn't feed your filters 'primordial soup'. If the sterile filtration's NOT part of your fill process, then there is not only no requirement to use nonsterile TSB, it's madness to use nonsterile TSB- it's a guaranteed failure.
What you put into your process simulation should be appropriate to what you're trying to validate.

A2: You are not required to use non-sterile media for your media fills because you should have your filters retention capability validated under your use conditions. The media fill is there to demonstrate your process downstream of the "clean side" of your filter does not impact the sterility of the end product.

A3: Thanks for all the feedback - the reason I asked is that we are being challenged that the use of non-sterile media upstream of filtration is industry standard, and that it is a requirement.

This came as a surprise to me, so I thought I would get some advice from all of you “experts"!

Some queries regarding

1)Why MacConkey broth needs to be incubated at 40 - 45 degree centigrade for
Ecoli,and what is the actual purpose of that?
Please explain the exact mechanism involved.

2) Why the industries are now preferring antistatic garments for aseptic
areas, what is the advantage of that,kindly explain in detail.

A1: Regarding question 2:

Garments with static will attract particulates (non-viable and non-viable with viable attached). Those particulates could then be transferred into your product during aseptic processing.

Also, depending on what chemicals are used in your process, garments with static could present safety concerns such as sparks. Most clean rooms are controlled with low humidity levels and some processes use solvents as drying agents. A spark could create a real problem.

A2: The incubation of MC broth at 42-44º is to select the growth of E. coli, but not other Enterobacteriaceae.
In my opinion this method could be excellent to test foods, in which E. coli is frequently present.
In pharmaceuticals the presence of E. coli is improbable, but the presence of other objectionable microorganisms that can grow in TSB at 30-35º could be more frequent, and the incubation in MC broth at 42-44º doesn't allow to detect them.

In our laboratory, we incubate in MC broth at 42-44º according to USP/EP only to avoid problems with auditors; but because we are not agree with it, we do in parallel a strike in MC agar directly from TSB to detect objectionable (but not specified) microorganisms.

Growth promotion test

Anybody can explain please how we do GPT(growth promotion test according to Canadian GMP &USP). Should we do the test on each sterilized lot or only once when we receive the media (either dehydrated or ready one). do we calculate by factor 2 ?I need all the references please.

Queries regarding GPT, pre-incubation and plate preparation

1) I expect most of the microbiology laboratories have the practice of pre-incubating their agar plates and broth tubes for 48 hrs at 30-35 degree centigrade is it OK or we need to incubate at 20-25 degree centigrade for Yeast and mould also, kindly clarify?

2) Does the pre-incubation for the sterility media i.e Tryptone soy broth ,Fluid thioglycollate broth and peptone, in case if it is mandatory where it should be pre-incubated and how long?

2 a) Tryptone soy broth - Temp /Time?

2 b) Fluid thioglycollate broth - Temp /Time?

2c) peptone - Temp /Time?

3) We have the practice of conducting GPT for the newly received LOT and only if it passes the test we subject the media for routine use,But during routine use (i.e after autoclaving the media) we perform the GPT in parallel to the testing, kindly let me know whether we are within the regulatory expectation or even in routine practice we need to wait for GPT result and thn use the media for testing?

4) How to avoid moisture formation occurring during the plates pouring any specific technique is available to prepare without the moisture formation?

A1:
1. 30-35 degree centigrade incubation will support both bacteria as well as yeast and mold growth.

2. If the holding time is validated for media sterility, media can be used after passing GPT. Negative control of the same media lot shall be incubated along with the test sample.

3. GPT passed medial shall be used for routine testing. This practice will be helpful for future investigation, if any.

4. Moisture formation can be avoided at the maximum by pouring the media at 40-45°C.

A2:
1) Answer: pre-incubating agar plates and broth tubes for 48 hrs at 30-35 degree centigrade is OK

2) Answer: pre-incubating all broth tubes for 48 hrs at 30-35 degree centigrade is OK

3) Simultaneous testing of GPT and testing of sample practice is also ok (If GPT fails, Enter test will be invalid).

4) Try to pour the plates at about 45 degree and after pouring, close the plates partially for sometime then close it completely.

A3:
1) Correct

2) Pre incubation is to ensure that the media is not contaminated . 48 hours should suffice.

2a): 20-25/ 3--35 ° c.
2b): 20-25/ 3--35 ° c.
2c): 30-35 ° C.

3): You need to perform for each lot of newly received Dehydrated media and also ; each lot of prepared media.

4): Invert the plates after solidification. Wipe.

A3: Its really good to see your passion about the microbiology topics, well let us start one by one to solve your queries.
1. If you are regularly monitoring your area where you are dispensing the media/broth and if you find yeast and mold count in the EMP plates then you should go for pre-incubation of plates for 20-25 degree also. Actually yeast and mold does not occur frequently in the clean rooms (If you are following the proper clean room practices) and during the dispensing or plate preparation human interference and environmental condition are more in concern hence we go for 30-35 degree to check bacterial contamination. Yeast and mold grow best at 20-25 but its not that it wont grow at 30-35 you can also get yeast and mold at 30-35 degree. Rest it all depends how you can justify your process.
2. yes this is also a growth promoting media and you should go for pre-incubation. What best you can do is incubate whole lot of media (TSB, FTM) for 48 hrs to check any contamination in the sterilized lot and keeping some representative tubes/bottle for further incubation from the same lot you can use rest of the bottle for testing. To justify the negative control for media you can incubate those representative tubes/ bottles for next 14 days in parallel with the sterility test. To incubate TSB and FTM for first 48 hrs put them at 30-35 degree and then transfer TSB at 20-25 degree for further incubation to mimic the sterility test. For peptone water (diluents) 48 hrs incubation is to be given for the lot and for representative bottles/flasks another 72 hrs so in total 5 days.
3. Yes you can do parallel testing with GPT because you might be mentioning about the GPT of the media in the product test results. If there is any ambiguity in the GPT or in the test whichever is not meeting the criteria you can repeat the test based on you finding otherwise you may report any false negative or false positive results.
4. You can use Automatic pouring and stacking machine where pouring is done at 45-55 degree centigrade and simultaneously cooled on peltier plates to 0 degree which avoid any moisture formation on the cover/lid of the Petri plate. If you don't have that machine then (I won't recommend it but it depend how aseptically you perform) what you can do is after pouring the media into the plates, spread the plates on the LAF working bench and slightly open the lid. When the media gets cooled(solidified) aseptically cover the lid and stack your plates in inverted position and proceed for pre-incubation.

stability testing for solid dose

To my understanding, solid dose product (such as Tablets, Softgels, Vitamins, etc) should not be placed on a stability program. Of course, microbial analysis is performed on the Finished Product for release but not placed on long term or accelerated studies........but 'x' costumer is requesting this to be done.

Any thoughts, comments.......does it make sense to place these products on stability testing since if there is no microbial contamination at time 0 then there should be no contamination at time x, y, z……..

A1: Having worked at solid dosage pharmaceuticals, I don't recall doing microbiology testing to stability samples. I am assuming that some possible scenarios are that (1)the manufacturer suspects that the product is getting moisture gain during storage and wants to prevent risks, (2) It is a new product so insufficient data is available to withdraw the testing requirement (3) product data shows risks or (4) the manufacturer's lack of knowledge/experience(??)has led to establishing the requirement.

The manufacturer needs to take a look at the products historic data (microbial) along with the products water activity results and any other microbial controls used at the manufacturing facility (i.e. Cleaning and sanitization procedures) and gather this information into a report justifying the change in product specifications. I am assuming that the reason the test is being requested is because it is in the product specifications.   These factors will help in demonstrating the effectiveness of the product towards microbial contamination.

Sterility of antibiotic susp.

How to validate the sterility testing of antibiotic suspensions for veterinary pharmaceuticals?

According the Ph. Eur. 6th edition, 2.6.1 (Sterility testing), the validation of antibiotic liquids is carried out with the membrane filtration.
The validation of oils en suspensions which cannot be filtrated, are carried out with the direct inoculation method, in which the preservatives are neutralized by an agent which has no effect on the growth promotion of the media.

There's no description about the validation of finished products consisting of antibiotic suspensions.
After you neutralize the preservatives, there's always one substance which contains antimicrobial activity: The antibiotic itself!

Of course, for the inactivation of penicillin we use penicillinase. We want to learn more about the inactivation of Cloxacilline, Gentamycine and Trimethoprim e.g.
Is there anybody who has experience with these antibiotics?

A1: Gentamicin, in common with the other aminoglycosides, is not amenable to chemical neutralisation. Its effect can be overcome by dilution in a suitably large volume of recovery medium; by washing on an appropriate membrane filter; or by enzymic inactivation.

The neutralisation of Trimethoprim is also covered in the chapter below.

Details can be found in 'Actual and Potential Methods of Testing Antibiotics' by Breeze and Simpson in Antibiotics: Assessment of Antimicrobial Activity and Resistance ed. by Russell and Quesnel, published by Academic Press 1983 pp 339-348.

A2: In autoclave validation sterilization cycle for media should be validated. Now in USP/BP/JP media should be sterilized at 121 degree temp. for 15 minutes. Now some companies sterilized for 20 minutes. Is it consider. B'coz USP suggest no over heat approach allowed.

A3: Media cycles should be validated as all other cycles are. USP does say '121C for 15 min'. This is also the manufacturer's directions for most media with the exception of very temperature sensitive selection media. However, USP also stipulates that the sterilization of 121C for 15 minutes is 'time at temperature'. In our cycles, it takes a validated time of 15 minutes for our media (for the volumes we use) to reach 121C. Therefore our cycles are 15 min. come-up plus 15 min. at 121C for a total cycles time of 30 minutes. If one is not sterilizing media 'at' 121C for 15 minutes you are not following manufacturers directions either.

Env. Monitoring in Laminar Flow Hoods

I know how y'all love benchmarking and I am in need of some information on industry practices for the monitoring of drug substance (API) procedures such as culture inoculations, open bulk filling ops and personnel practices performed in production laminar flow hoods or bio safety cabinets. I am looking for feedback from Biologics drug substance manufacturing companies. Any responses would be greatly appreciated. If you could also add what market your product serves (US, Europe, Asia, Japan.)

1a. What environmental monitoring is conducted in Laminar flow hoods used for production culture inoculations and other support activities?

Viable
Non-Viable
Settle Plates
Working surface contact plate
Personnel

1b. When is the sampling performed? pre-operation in operation (continuously or a pre-determined intervals)

Post Operations
Other?

1c. What is the monitoring frequency?

2a. What environmental monitoring is conducted for DS open bulk filling operations?

Viable
Non-Viable
Settle Plates
Working surface contact plate
Personnel

2b. When is the sampling performed? pre-operation in operation (continuously or a pre-determined intervals)

Post Operations
Other?

1c. What is the monitoring frequency?

Sterility Assurance Level

In choosing SAL level, factors are determined as 10-3, 10-6, 10-12, why intermittent values like 10-4, 10-5, or 10-7 was not chosen. Is their any statistical theory behind on this? Why its been doubled?

A1: SAL should be chosen by the organism that you are trying to get rid of. Pharmaceuticals uses 10-6 because they want to decrease the risk to 1 in a million chances. The food industry uses 10-12 because the target organism is Clostridium botulinum. If you have a pure spore suspension of C. botulinum the maximum number you could have would be 10-11 spores. So 10-12 is 0.1 spore left, less than one. For milk and eggs Salmonella is the target organism and 10-5 or 10-6 is used because it must decrease the number enough to not cause disease. For some salmonella you could have 10 CFU per dose and get ill. That is rare but has happened.
For SAL levels you want to end up with 0.1 organism or less than 1.

A2: Historically the 10-6 SAL value has been the SAL of choice and usually the required SAL for sterile medical devices. This selection of 10-6 was not based on risk analysis and safety, it was chosen in the U.S. because it was previously chosen by the Swedish in the 1960s and everyone else thought it sounded good. The "one in a million" probability corresponding to 10-6 seemed to provide enough safety for medical devices.

In the U.S. SALs of 10-3 have been widely accepted for topical medical devices which are not intended to come into contact with compromised tissue and for in-vitro medical devices, but this is not always accepted internationally.

It has never been required or requested to use 10-12, nor is it common to do so. It is common to use a 12-log reduction in most sterilization cycles (except for radiation), but a 12-log reduction is very different from an SAL of 10-12.

Microbiological Validation of PAA based SIP

While having worked for considerable years with vaporized H2O2 as a sterilizing agent for aseptic fill SIP (Sterilisation in Place), I am now involved in a project involving Wet Peracetic Acid (PAA spray) as the agent for automatic SIP of an Aseptic Filler Machine.

My basic question is What is the general consensus out there for BI validation work,. Unlike Vapor or Dry Gas systems, in theory the washing effect ostensibly ensures little meaningful recovery of residual spores from inoculated coupons or packaging component surfaces, traditionally common practice for VHP systems.

In discussion some colleagues have reckoned that whether the spore titre is being washed off and out of the system or killed in place is a moot point but I'm a little hesitant to adopt this rationale.
From my own perspective, I'm tending towards trying to verify kill in lab based studies using worse case exposure and concentration conditions and in addition then relying on physical / chemical verification of suitable dispersal and coverage of PAA on critical surfaces/pathways.

I would be grateful for any feedback from those with experience with PAA -SIP systems and any relevant references to papers, case studies or methods including physical/ chemical tests for PAA presence, PAA neutralization and ways of preventing wash off, (if any exist). I'm anticipating that maybe [name redacted] or [name redacted] from Raven / Mesa will have some interesting contributions to make.

No Increase" criteria for USP and EP

I'm trying to understand the "No Increase" criteria in the European Efficacy of Antimicrobial Preservation Test (5.1.3) What is "No Increase" at Day 28 relative to? The previous plating result? Or the original inoculum level?

I realize that it says "against the value obtained for the inoculum". It seems to me that this would allow for the required 1, 2, or 3 log reduction at the previous plating and then quite a bit of rebound growth, and the sample would still pass. Am I reading this correctly?

I am more familiar with USP criteria for passing the Anti-microbial Effectiveness Testing <51>, which includes a "No Increase" criteria at Day 28. This, however, varies with reference to bacteria or yeast & mold testing. For bacteria, "No Increase" at Day 28 is relative to the Day 14 plating, but for Y&M, "No Increase" at Day 28 is relative to the original inoculum level. These criteria are fairly clearly worded.

Anyone have sure interpretation for this in EP?

A1: My view on this is that:

1) "No Increase" refers to the previous reading point;
2) "Log reduction" refers to the inoculum level;

I have heard, that EP is planing on posting a Q&A section on this Test

preservative efficacy testing

Our company has manufacturing antimicrobial oral products having capacity of 240 ml.for the analysis of preservative efficacy test as per USP, how much sample is required? Otherwise need to perform the whole content or not? plz give the suggestions.

Handling Positive BacTAlert Results

I have a client who makes reagents that are used in the blood industry. They do not claim sterility for the products but make every attempt to manufacture a sterile product. They test the product with a BacTAlert system (qualitative test). When/if they get a positive result it is investigated just like a sterility positive would be done and they allow retesting on double the original number of units (as used to be done for sterility testing in the old days). Has anyone seen similar situations? If so, has anyone experienced feedback on such practices from regulatory bodies?

A1: Not all contaminants generate carbon dioxide - so I hope these folks gave validated this technique's application.
Not sure of the regulatory implications but this approach is technically wrong. As has been discussed repeatedly and extensively, retesting without justification does not impeach OOS results and statistically "double the original number of units" is inadequate.

What is their actual specification?

Water system validation

Purified water system validation Phase-I and Phase-II time period is 2-4 weeks for all sampling points, now any guide line we can perform the Phase-I perform only one week.
Pl. provide related guidelines.

A1: These are the FDA guidelines to the Inspection of High Purity Systems. It provides brief requirements on the validations phases and time period:

http://www.fda.gov/ICECI/Inspections/InspectionGuides/ucm074905.htm?sms_ss=email

A2: Your approach for sample frequency certainly seems sound. Here are other points to consider and address:

prerequisite: qualification of all test and sampling materials
qualification of the system hardware (IQ, OQ)
training and qualification of technicians
validation of test methods and sample volumes
validation of sample handling
establishment of written PM requirements for the system
inclusion of any routine sanitization processes in validation
establishment of trend analysis requirements
use of microbe identification
inclusion of any corrective actions in the event of a problem
use of historical data to set preliminary alert levels

A3: As mentioned, you already have More than 8 years of Data of both TOC as well as Microbiological Quality of your system.

Now if you are concerned about validation of your system, please first focus on the term validation. As per any regulatory or FDA itself, the validation comprises three types namely:

1. Prospective- Validation you have to conduct for newly installed system.
2. Retrospective- Based upon the historical data, you prove that your system is performing satisfactorily.
3. Concurrent: - In which you validate your system with ongoing process.

If you are having 8 years of data to prove that you are consistently gaining the water which is meeting the Quality of Purified water, I don't think so, that you have to perform the whole validation for your system. However if you want to ensure the consistency of your system a week data of all the points (Sampling as well as user Points) is more than enough.

A4: All I can say is for you to let logic prevail over paradigm. If you have 8 years of data, albeit at haphazard times during the week, you have a lot of data. If it all passes or at least has explainable/ correctable deviations covered by CAPAs and/or truly attributable to sampling or testing error, then you have a great deal of justification that the water system is operating under control. Aside from the necessary IQ and OQ data for the purification components to baseline the system and show that each unit op is working, you may have close to all you need to demonstrate consistent and continuous control as well as efficacy of sanitization efforts.

However (and there always is a "however"), the manner in which the POU sample has been collected can skew the test data if the samples are not collected EXACTLY the same way that outlet is used -- same hose, same flushing procedure (if any), same outlet sanitization procedure (if any), etc. If not exactly the same, you are deluding yourself if you think that is the quality of water that is being delivered for use from that single POU if the sample is collected with a sterile hose, no hose, or the manufacturing hose that is exhaustively flushed for sampling but not so flushed under normal use.

If the sampling has been different for testing than for regular manufacturing use or if manufacturing use (when you get your POU samples) tends to not be as frequent on say Mondays and Fridays as on Tuesday through Thursday, then you need to do daily sampling as you have outlined. Just keep in mind that the purpose of validation is NOT to adhere to some arbitrary sampling plan but to show consistency and reliability.

Let logic rule. That means that you will have to explain your logic for why you historic testing is just as reflective of consistent and reliable quality as daily sampling during this validation. Explanatory words on a written page are much cheaper than reams of data that then also have to be explained. If you have trouble making this logical point in writing, then you may need to fall back on a standard sampling plan. My vote is for a logical discussion every time over mindless, short term and relatively meaningless test results on inappropriately collected samples.

environmental control during antibiotics production

We are trying to find a good method for environmental control inside isolators when working with an antibiotic of the aminoclucosides group, but we can not find any culture medium with inactivating agents specific for this type of antibiotics. Any suggestion??

A1: Have you considered using one of the rapid micro methods for environmental monitoring in your isolator? This would eliminate the burden of using culture medium. One question: does your manufacturing conditions produce significant particles in the isolator? This may influence the types of rapid methods you could utilize.

A2: Why must we conduct microbial monitoring inside an isolator?

A3: For good or bad, most regulatory authorities expect to see monitoring within an isolator environment, irrespective of the demonstrated sterilization of the contents by H_2O_2 or peracetic acid vapor or whatever. Normally this is pretty limited- some settling plates and RODAC's, also particulates and active air sampling for manufacturing operations (like filling/stoppering) conducted within isolators- but it IS an expectation.

A4: We've got a lot of powder in the isolator chamber because we mill and micronize the product inside it. This is the reason why we can not use routine environmental control (active air sampling), given that the agar plates are covered with powder during the sampling.

A5: The isolator has two transfers at the entrance and at the exit. The number of openings is very limited and it is located in a room classified B, so the risk of contamination is very very low, but anyway we must confirm the environmental quality inside it.

antibiotics microbial assays

I must determinate potency of a sodium fosfomycin for injection. This antibiotic is not reported in USP 32, only in Japanese pharmacopeia, but the Proteus mirabilis ATCC 21100 that is used is not available in our country (I'm in Argentina). There is another alternative? What is the problem if I use another Proteus as ATCC 7002?

A1: What specification are you claiming for the material and product containing it? If it is JP then you need to follow the procedure in this case. You cannot just exchange organisms without confirming that they both perform in the same way in the assay.

Aseptic technique and Sterility Testing

Does anyone have any good references on Aseptic technique with a section on performing a sterility test? Aseptic technique may be highly subjective. I have my own standards, but how do you enforce these standards if auditing?

A1: I wanted to ask what kind of laminar would you suggest for sterility testing of samples.

Information transfer

I was discussing with some fellow microbiologist lab managers recently on the issue of keeping current with the science, regulations and new technologies. We all agreed that we did not have as much spare time to "read around" our speciality as we would like due to the intensity of our roles.

When looking for specific information then we all went to the internet and "Google" the topic, but the method of picking up new ideas etc seemed to be from various sources. Most picked up new info by attending conferences or from people that had been to a conference. Some read the email marketing from suppliers but most put it to the "later to read" pile and never did. Some read the trade magazines and picked up ideas from the adverts or articles.

I am interested to know if people feel they have enough time to keep up to date and if so what route they find the most efficient to find relevant information? For those with not enough time do you see that as an issue?

A1: This is my view, Yes in the pharmaceutical industry laboratory's the work is never finished and the work does not seem to afford enough time to read around one's area. If one is an analyst or a technician whose job is to produce high quality data under guidance, one may be able to get away with just doing that. But if your job requirements includes providing guidance, there is not excuse for not finding the time to do the extra bit of reading. If one has to drop a meal to find the time one has to do so. If one has to cut sleep hours one needs to. If one needs to stay an extra hour then do so. Current technology has made life much easier. The problem is that we have become lazier. As you suggested, there are a number of free sites that one can get peer reviewed materials from. Many manufacturer's provide excellent technical information and scientific back grounds. This is not going to help any if one could not muster the discipline to just sit down and just go at it. If we can give one our a week to "Lost", "24" or even "The bachelor", six hour to football, 1 hour to birthday parties... shouldn't we try to give at least 2 hours a week for some

thing that our livelihood really depend on??? It might also help to infuse some "academic" atmosphere in the work place. Why not have "advances in pharmaceutical microbiology" seminars, talks, or journal clubs say once a month organized by your team? So lets throw out those excuses of being overworked and not having time and just march with times!

A2: Suggest pubmed @ http://www.ncbi.nlm.nih.gov/sites/entrez?SUBMIT=y
google scholar http://scholar.google.com/

Searching the general google is a waste.

A3: You are likely to provoke comment on this.

It is a real problem though - many of us are too busy to keep up with the literature which includes trade journals, scientific journals, regulatory documents and perhaps even vendor manuals. Just finding the relevant articles can seem daunting.

However, it is critical that the micro lab is sharp and this will depend on the manager or department head. He must be the subject matter expert and he must be current. I really do not want to open up the "who is a microbiologist" topic again, but it is amazing how much of the success of the lab depends on the leadership.

To your point. There are several sources for some good info. PMF in the US is one (http://www.pharmaceuticalmicrobiology.org), the Microbiology Network (http://www.microbiol.org) and its webinar series is another (https://micronetwork.webex.com/ - especially check out the recorded sessions during the summer lull). Truth in advertising; I am deeply involved in both these sites.

Having urged the conferences and webinars I will continue with the observation that while they are helpful, they are not sufficient to keep you current. The bottom line is that there is reading that needs to be done, even if you find the good conferences and the accurate webinars.

One service that might be of interest is a reading service that compiles lists of articles of interest for the manager, who then indicates the articles he would like to review. This allows the manager to focus time on reading the articles rather than searching them out.

A4: Actually Scott, there is a great lit service. It is the PMF newsletter that was issued a few months ago. I am still going through many of the articles sited there.

A5: You might want to look into a subscription to "BioQuality." A monthly newsletter on quality related issues.

Good concise source for regulatory and quality related info. Each month they present recent 483 observations. Good listing each month of recent guidance documents & published articles.

A6: I wish I had more time to read all of the microbiology articles that come across my desk and to be able to search the web for recent advances in pharma micro! In addition to what Scott had suggested for online resources, a number of pharmaceutical microbiology websites are now offering blogs and news pages that make it a little easier to keep up with the current trends. For example, Tim Sandle has an excellent blog on his Pharmaceutical Microbiology website (http://pharmig.blogspot.com/). Tim does a fantastic job of posting information of pharma microbiology interest on almost a daily basis. I also publish a blog that is specific for rapid microbiological methods (http://blog.rapidmicromethods.com/). Both of these online sites also provide information on recently published books and papers that are relevant to the pharma microbiologist. And if you really want to cut down on the amount of reading and only focus on the "headlines", both of these sites also publish their blog on social networks such as LinkedIn, Twitter and Facebook. If Pasteur had an internet connection I am sure he would be following the discussions!

A7: I recently read the following in a mail from one of my LinkedIn groups and think it may be relevent to this discussion: "Invitrogen has developed a new Life Science on-line search tool called Select. It has been designed to significantly reduce the time spent searching on-line for the latest papers and developments. Select uses unique RSS spidering technology to constantly monitor over 200 Life Science journals and libraries, including PubMed, Nature, Biocompare.com, Bionity.COM and Cell, to name but a few! Users can define their own search terms and subscribe to any number of journals from within the Select Library. Any matching papers and articles are compiled into a simple daily ebulletin, featuring a short abstract from each paper plus a link to the full text. The Select service is completely free to use - no matter how many sources you subscribe to, or how many search terms you want Select to monitor. To register for Select please click on the link below (or paste into your browser), http://snipurl.com/select104

It takes about 5 minutes to set up your preferences and you can change these at any time

If you have any feedback about Select – both good and bad, I would love to hear about it. Please feel free to post your feedback here or email me directly at andrew@invitrogen-select.com"

I haven't used it yet but maybe some of you would be interested in giving it a look.

A8: Of course, PMF newsletters are a great resource. There are many other resources which has been already mentioned by other contributors.

I think, what [name redacted] meant by posing this question is, in the limited time available, how does one devote time and get up to-date with all needs simultaneously. As Scott writes, to become a 'subject matter ' expert in this and any field of microbiology, it takes time!!! And if one wants to become an expert, there is no excuse, one has to find time as Phil writes! I agree with him and others. Nothing comes quickly!

A9: I came across this site a few months ago, it may be useful to some.

http://www.bioqtforum.com/index.html

Methylobacterium mesophilicum

Could anyone tell me if Methylobacterium mesophilicum can utilise carbon from isopropyl and ethyl alcohols in the same way as it can from methanol?

A1: Yes it will grow on diluted ethanol.

See http://ijs.sgmjournals.org/cgi/reprint/38/1/124.pdf

A2: Ethanol yes, I think isopropanol as well

Sterility Test Result OOS

I have an OOS for sterility test result at the 5th day we found turbidity in the tube (canister) the product is injection 1mL I sent the canister for identification, but there's no result yet.

Did anyone had a problem like this before? How should I do the investigation Resampling have been done for another 40 sample, and we did the sterility test and the result is negative.

A1: I assume this positive was in an aseptically filled product, and not terminally sterilized? Growth on day 5 indicates a small inoculum that took several days to grow out. Hard to say if it's an true contaminate or inadvertently introduced by someone during the process. Follow USP <71> guidance on this one, knowing that the sterility test in this situation has little efficacy in detecting a real contamination.

Validation of software used for trending and setting specs

I wanted to get some industry feedback in order to determine if companies are generally validating software (SlimStat and JUMP) which is used to trend in process and release testing as well as setting specs.

Rapid micro bio system-

I am currently evaluating rapid micro systems and I would like to know if anyone has used and could comment on their experience with the Growth Direct system from Biosystem or any other system that could provide similar or better performance. How much additional validation work was needed in addition to that conducted by the vendor?

A1: You would be expected to follow currently available recommendations for validating your rapid micro system as provided in PDA Technical Report #33, USP <1223> and/or Ph. Eur. 5.1.6. Although each of these documents suggest that the end-user can use the vendor's data for robustness and ruggedness, you should be able to conduct testing to demonstrate that you are meeting the acceptance criteria for other validation parameters, such as accuracy, precision, limit of detection and quantification, linearity, specificity, etc. Additionally, if the vendor has submitted a Drug Master File (with the FDA), the amount of testing you would have to conduct <may> be reduced, but this depends on the data that was submitted and if your application and test samples are similar to what was evaluated by the vendor. As for whether the Growth Direct system or any other RMM technology is appropriate for your specific application, this will be determined by your test material(s) and compatibility with the RMM method, your required level of sensitivity, the types of organisms you will need to detect or enumerate, desired level of automation and throughput, cost per test, etc. There are a number of published references you can find from end-users who have validated RMMs in their facilities, as well as discussions on different types of rapid method technologies for comparative purposes. I provide a comprehensive list of rapid method validation and technology articles on my website (http://rapidmicromethods.com/files/references.html), and Scott has provided an annual overview of published microbiology papers, which includes RMMs, in the PMF newsletter (http://www.microbiologyforum.org/). There are members on this list who have validated the growth Direct for their companies, and hopefully some of them can provide specific information on their experiences.

Environmental Monitoring Benchmark

I want to know what is the industry practice in terms of the frequency used for Environmental monitoring of rooms and equipment in non aseptic facilities (solid dosage form). I have seen companies that monitor the locations once a year , other twice a year, quarterly etc. What is consider acceptable as a minimum? When should this frequency be increased? Your comments on industry practice and criteria's on how to define an establish the frequency is greatly appreciated.

A1: For bench marking my facility does a year of testing. They want to know the seasonal microbial flora that is present. Based on this data, we adjust our alert and action levels. The aseptic facilities generally do it every 6 months partly because they have a shutdown period where as my non-aseptic facility does not.

Reduction of environmental monitoring samples

Regarding environmental monitoring at sterile facility we are planning to reduce sampling points (settle, active air samples, total particle counts, contact and personnel samples) as well as frequency, if possible, in order to remove meaningless samples (we have data from several years experience). Our idea is to perform a Risk based strategy. Does anyone know any guideline to follow for this purpose? I would

appreciate any piece of advice based on personal experience in order to stablish the appropiate criteria for this task.

Regarding Clostridia

I would like to know that is it required to do test of Clostridia as specified microorganism in water monitoring?

A1: it is not mandatory to do clostridia test, even specified microorganisms also not part of the USP/EP and chances of anaerobic organisms in water is very less.

Skills Valuable after a Layoff

John Challenger, CEO at outplacement consultancy Challenger, Gray & Christmas, says in a phone interview that pharmaceutical layoffs are of greatest concern to those who are midway or late in their career--those in their late 40s, 50s and 60s. Their high income level and mastery of skills that are less in demand today than when their careers began conspire to make future placement difficult. Unlike their younger colleagues, they'll have more difficulty adapting to an environment of changing technology.

But those with fungible skills, regardless of career stage, have additional options open to them. "They don't have to stay in pharma manufacturing," he says of those who work in inventory control, purchasing and finance, for example. "They often move to tangential industries and continue to work in their area of expertise."

My question is it true that adaptability is largely dependent on age or other presonal attributes?

My understanding is you need 10,000 hours of practice to master the skills of your profession so this would mean you need to be in your 30s.

another CCIT question

For those who are using Dye Ingress, what is the detection limit of the Methylene Blue are you claiming?

Lactobacillus acidophilus recovery

In some testing, we are achieving slightly less than expected recovery of this organism in a probiotic product that also contains vitamins. It is suspected that the vitamins are inhibitory. We currently test under anaerobic conditions using MRS media at pH 6.5. We are considering lowering the pH of the media and also wondering if any of the common antimicrobial neutralizers might have any effect on the inhibitory properties of the vitamins, if indeed they are inhibitory. Any advice would be greatly appreciated.

A1: Hi. Can you add the mixture to fluidD and filter the mixture through a 0.45 micron filter, rinse the filter with fluidD and then place the filter on a MRS plate? I would try that. The vitamins will be removed.

You are comparing the recovery, of the Lactobacillus acidophilus with Vitamins, to a culture on the same media (pH) without vitamins (and same anaerobic growth conditions). So I am not sure why lowering the pH would help. What is the optimal pH for Lactobacillus acidophilus (Bergey's manual should have it)? Sodium azide is used a lot to isolate lactic acid bacteria if there are competing organisms. Sodium azide kills anything with a respiratory system (lactic acid bacteria only ferment).

Anaerobic growth may not be optimal for Lactobacillus acidophilus. Most lactic acid bacteria are microaerophilic. The like a small amount of oxygen. They lack the enzyme Catalase, but still have super

oxide dismutase, so prefer small amounts of oxygen (up tto the point the kill themselves by making hydrogen peroxide). I used to grow lactic acid bacteria in an anaerobe jar (no anaerobe gas mix), but add a small candle to the jar, light the candle, put the lid on the jar, and Let the candle burn out (without causing a fire). The end result is microaerophilic environment (did this in a research setting). Things grew pretty well. MRS medium is pretty standard.

A2: [name redacted] try using the stomacher for 1 min. at 230 rmp. Also add L-Cysteine to the MRS. Use Difco brand vs. other brands of media. Is that the only strain you have in the vitamin?

A3: Those questions would appear to be open to answer by experiment. I'd look for an answer in data.

Standardised Suspension Of Aspergillus Spores for PET testing

Does anyone know if and where can I purchase standardised suspension of Aspergillus niger spores for PET testing.
I may have a problem with testing due to clumping of the spores from the in house preparation.

A1: You can try BioMerieux

A2: Bioball, which I think you can get from BioMerieux, go up to 10*8. I’m not 100% sure they do Aspergillus brasiliensis as I haven't used them.

Have you tried using 0.1% Tween in your suspension medium when making the in house spore suspension?

A3: We have recently bought calibrated lyophilised pellets of Aspergillus brasiliensis for PET - Epower (Mean Assay Value : 1.0-9.9E+06 CFU per pellet)

Please check the "Microbiologics.com " site

A4: One suggestion for breaking upon clumps and clarifying the spore suspension is to filter through several layers of sterile cheese cloth. Also, I assume you are using a detergent solution like 0.05 % Triton X100 to prepare the spore suspension.

A5: Have you tried adding tween 80 to your in house preparation? This can prevent clumping.

A6: Check out Microbiologics web site. They have EX-PEC and E-Power products, specially designed for PET test.

A7: A drop of detergent, such as Polysorbate 80, in your suspension solution may overcome clumping.

A8: BioMerieux does offer A. brasiliensis in BioBall 10e8 format , as well as C. albicans, E. coli, P. aeruginosa, and S. aureus

A9: If by PET you mean antimicrobial preservative efficacy testing, then the spores have to be of a certain age (I believe 7 days old), and can only be maintained under refrigeration for one additional week. The guidelines are pretty specific about it. So I would assume that purchased preparation would not be acceptable, unless somehow validated. but then PET could be an abbreviation for another type of test as well…

A10: Yes some of us in New Zealand call Antimicrobial Preservative Efficacy Testing PET - short for Preservative Efficacy Testing.
The challenge I was experiencing was that the test was failing at 14 days for Aspergillus and the client was concerned it was due to a spores sticking together. Their request was to obtain a commercial standardised suspension in order to avoid any false fails.

I have sourced the appropriate culture ,but decision was made to follow the guidelines and use 7 days old culture, which I will be using tomorrow after filtering it through the sterile microscope lens tissue and adding Polysorbate 80.
I will establish the numbers by microscopic count and a plate count as well.

A11: I do not see how the recommendation from the client that the use of a commercially prepared Aspergillus brasiliensis spore suspension would prevent the spores from sticking together. By the spores sticking together, I would think that the counts would be less than if the spores were not sticking together. The use of either Tween 20 or 80 at a concentration of 0.05% or Triton X-100 (Octoxynol 9) at a concentration of 0.1% in the microbial count diluent would prevent the spores from sticking together. If the counts of Aspergillus brasiliensis were not stasis or reduced in the test sample at the 14-day sampling point, I would think that the formulation was inadequately preserved if there was an increase in the 14-day count from the theoretical inoculum count in the test sample. Furthermore, I do not think that the use of sterile microscopic lens paper would be sufficient for your purposes in obtaining a spore suspension without mycelium fragments.. Generally, I would recommend that you use sterile glass wool that has a porosity between 40 to 100 microns to obtain a harvested spore suspension. I have even seen people use sterile gauze as a filter to obtain spore suspensions.

A12: ISO 14729, Microbiological requirements and test methods for products and regimens for hygienic management of contact lenses, recommends to filter the challenge fungus (in this case, Fusarium solani) through sterile glass wool, cheese cloth or gauze to remove hyphal fragments. This may also help to remove clumps of spores from the suspension. The ISO standard also states that the bacterial suspensions may be filtered (e.g., 3 to 5 micron pore size) to produce a single cell dispersion.

A13: We used to filter the Aspergillus spore suspension through sterile gauze pads placed in a sterile filter - you could also use cell strainers which are commercially available.

Software inquiry for calculation of Fiducial limits / confidence interval for microbiological assay USP

I want to know about the suppliers of software for calculation of fiducial limits / confidence interval for microbiological assays as per USP. Could any of the forum members help me and provide feedback in case of the ones that are being used in the industry.

Re-Mapping of Micro Incubators

Question Re: re-mapping of micro incubators that have undergone IQ/OQ/PQ and are continuously monitored for internal temperature by an independent qualified system.

Is there an industry standard or requirement for the Pharmaceutical QC laboratory as to how often a microbiology incubator should be remapped for temperature uniformity?

What types of non-scheduled maintenance or repairs would warrant that the incubator be remapped for temperature uniformity?

Since this is a re-qualification / re-mapping, is a partial or loaded incubator remapping acceptable as this would be the worst case scenario?

A1: We re-map all of our controlled temperature units (incubators, refrigerators and freezers) annually. As we are a contract testing lab, we have to exceed our clients expectations when it comes to GMP and quality. I am not aware of any re-mapping requirements in the regulations, other than showing that we maintain strict control over our critical equipment. As for partial or full mapping, we specified in our SOP's that the units are simply tested with whatever materials are in them. That will save you from artificially removing or adding material to meet your requirements. Our CTU's are monitored by a Kaye LabWatch system.

A2: I missed your inquiry, but "ditto" to [name redacted] comments. After initial IQ/OQ/PQ our instrument qualification SOP requires annual PV (performance verification) to show that the instrument is still performing as originally qualified. Again I am not aware of any regulatory requirements, but our clients as well as FDA investigators are very pleased that we include annual PV's on all instrumentation related to GMP compliant analysis. At the previous company where I worked (pharmaceutical manufacturing facility) they too had the Kaye LabWatch system which is quite sufficient for independent monitoring albeit a lot of "fun" to qualify!.

A3: Mapping of incubators has been extended from Stability Chamber mapping practices, another in a long slippery slope where things become the expected norm. At its core...you need to have qualified equipment that consistently performs to predefined performance requirements pertinent to the test, process, etc that is being performed by said equipment. This is at the core of fundamental cGMP. You should have a scientific and engineering rationale justifying your approach to maintaining accuracy, precision, performance, etc. of all your equipment and be able to defend it.

SMA Validation Services

Can anyone recommend and provide information on a testing organization that can perform a validation on an air sampler according to ISO 14698-1?

A1: Biotest performs ISO 14698 testing for RCS Air Samplers.

Lactobacillus culture maintenance

CAN anyone share any experience in propagating and maintaining / long term preservation of lactobacillus cultures such as lactobacillus sporogenes, lactobacillus acidophilus, lactobacillus paracasei. should i go for glycerol stock preparation or should i store them in 20% skim milk solution as was mentioned in some reference articles. Kinldy guide me with a protocol for the same.

Pyrogens test

Does someone know wich is the dosage that must be inject in rabbit for the pyrogens test? Is it the clinical one?

A1: Normal dosing is 10mL/kg body weight, unless the USP monograph or other regulatory commitment documents indicate otherwise.

A2: The major pharmacopoeias have issued guidance recommending the elimination of the pyrogen test either as a matter or course or where equivalence can be shown against LAL or MAT (following the recent inclusion of the Monocyte Activation Test into the EP). Until 2009 with the EP, blood products were excluded from a path towards any alternative test to the pyrogen test however the forthcoming 7th edition now allows for the LAL test to replace the pyrogen test.

There are some who would argue that the LAL test is not a direct replacement. This is based on the LAL being a specific assay designed to detect bacterial endotoxin whereas the pyrogen test, as a non-specific test, will detect other pyrogenic substances like exotoxins. On the other and, there is a reasonable amount of research which argues that endotoxin is the most ubiquitous pyrogen in pharmaceutical manufacturing and that other pyrogenic substances are rarely found or found in such low numbers that they are below the pyrogenic threshold.

Where a pyrogen test is required, and an alternative path has not been sought, this should be specified in a pharmacopeial product either for the product of for the active ingredient. If it isn't, then I would make the case to develop the LAL test as an alternative should a test for 'pyrogens' be desired.

Such a move saves time, money and avoids the unnecessary use of animals.

A3: As long as I know from my experience as LAL Test distributor in Argentina, the Pyrogen Test was replaced by the LAL Test.
Only a few products that couldn't be validated by the gel clot method are tested by the pyrogen test in rabbits.
The gel clot method is the most used test in Argentina.
The photometric methods like the kinetic method in tubes adopted by some companies in Argentina is more sensible and let the user increase the Maximum Valid Dilution and test and validate all their injectables.

Cleaning Validation Protocol for Ophthalmic Drops

Would you please help me sending a Cleaning Validation Protocol for Ophthalmic Drops.

Elimination of SDA surface plates from EM

I am trying to qualify TSA rodacs for exclusive use in surface sampling, i.e. no more SDA rodacs. My protocol currently calls for acceptable growth promotion using ATCC organisms plus some environmental fungi. It also calls for acceptable recovery from surfaces (this was meant to be an ancillary portion to support a standardized sampling technique), and acceptable recovery from surfaces for TSA in comparison to SDA. Most of the results passed (except for the recovery efficiency from surfaces, which further study has shown it to have unrealistic acceptance criteria), and I think I have figured out the issues with the rest. Has anyone done similar studies to eliminate the use of SDA and/or to provide a standardized surface sampling technique? Are there any criteria beyond what I have mentioned that should be expected? Any help is appreciated.

A1: I am pretty sure the EU wants you to use SDA RODAC plates and TSA plates incubated anaerobically at least twice per year.

You can do this by double monitoring (TSA and SDA also TSA & TSA anaerobe jars) once every 6 months.

A2: We did a retrospective study showing that everything we had recovered on SDA @ 20-25 we had also recovered on TSA @ 30-35.

A3: What kind of study did you do? I am trying to eliminate quarterly SDA monitoring from our EM program. Using SDA (for viable air and RODAC) has provided no additional value whatsoever. Except now that it's in the SOP, we have to justify its removal. This method is merely suggested in the USP, which does not necessarily mean that it must be done.

A4: We did 2 things, a data review that showed that every species we had recovered on SDA we had also recovered on TSA, and a formal study using TSA and SDA both incubated up to 7 days at both 30-35 and 20-25 with normal growth promotion organisms and some environmental isolates. These was no advantage to the SDA at 20-25 for 7 days over the TSA at 30-35 for 3 days. I will add that this is true for OUR in-house flora. It may not be true for yours. Also see:

Marshall, Poulson-Cook, Moldenhauer, *Comparative Mold and Yeast Recovery Analysis (The Effect of Differing Incubation Temperature Ranges and Growth Media)*, PDA Journal, Vol 52, #4 Jul-Aug 1998

A5: This is funny in that everytime the subject comes up, I see countless studies people have done to prove this practice isnt really value added. I think you should all come together and write a book.

Seriously! There seems to be a lot of data that has been done that would be great to pull together in some form of paper. At some point the data needs to speak for itself in refuting some of the misconceived practices that took off like wild fire because someone once had too much time on their hand and wanted to add excess fluff to cGMP.

A6: You are right - there really is no need for SDA. SDA is designed to suppress bacteria with its low pH (5.6) while allowing the more acidotolerant yeast and mold to grow. There really is no need for this given the low numbers we commonly see on EM plates. Makes sense in food testing, not so much in clean rooms. One modification to TSA that you might want to try is to supplement this with 0.5% glucose (helps the yeast and mold!).

A7: I am confused the more I ponder over the explanation given on the SDA with supplement [antibacterial] added, because of the following reasons
1. considering the temperature we incubate for bacterial [30-35 degree celsius] that do not favour mould and yeast at [20-25degree celsius] then one will want the SDA medium to be considered.
2. the acidity of the {TCA medium } used for bacteria isolation do not favour the yeast and mould so SDA will have been the best choice ,
3. The period of incubation for bacteria will be between 24hours at maximum for 48hours , this would not be enough for the growth of mould by the time one id reading the TSA plate .
4. Also the sugar concentration of the SDA medium mould and yeast can tolerate better than the bacteria.
When I checked these reasons I consider the use of SDA more relevant to the environmental studies even if the TSA will be used for the same number of days and same conditions.

A8: Suggest that folks' reports of successful validation for TSA recovery of fungal isolates reveal non-permissive factors of that medium vs. fungi are overblown - esp. in the case of the usual fungal suspects of Aspergillus and Candida.

Plenty of bacteria grow at pH 5.6 and plenty of fungi grow at neutral pH - through either temp range - and one would presumably miss fungal or bacterial acidophiles on TSA. The dextrose concentration is certainly not a limiting factor - tho as Scott said, addition might help fungal growth rate. The only effective consideration might be growth rate and again, there are plenty of fungi that would be seen within 48 hours. Those that would be missed would probably include be those looking for a lower Aw environment and they wouldn't find it on SDA either.

What you may see is a change in fungal colony morphology on TSA v. SDA (color, rate/extent of sporulation, etc).

Maybe, as [name redacted] said, those claiming validation (and those claiming otherwise) should publish.

A9: (1) Marshall, Poulson-Cook, Moldenhauer, *Comparative Mold and Yeast Recovery Analysis (The Effect of Differing Incubation Temperature Ranges and Growth Media)*, PDA Journal, Vol 52, #4 Jul-Aug 1998 Carole

GPT

Is valid to use Miles Misra method to do medium GPT?

A1: Why wouldn't it be? Do you get valid counts from the method?

A2: Actually Miles Misra was one the techniques that our original ISO accrediting body (UKAs) recommended for the lab. We are using the current USP GPT mentioned in <61> and <62> and have expanded that to all of our media.

soap solution used for washing glassware

Which soap solution would be suitable to wash glassware ? Is it required to carry out the washing cycle studies to find out the ppm levels of the remains of soap solution after washing? If yes, what is the method followed?

A1: In general, the detergent(s) used for cleaning glassware should be effective at removing the broad spectrum of soil(s) that may be present. Most organic soils can be successfully cleaned with an alkaline cleaner while inorganic soils are addressed by acid-based cleaners. In addition, the cleaner must be free rinsing, low foaming, substrate compatible, and if applicable, hard water tolerant. If this is a cGMP application requiring cleaning validation, then it is important that the detergent be selected from a supplier that assures formula integrity and supportive documentation.

The acceptance criteria for cleaned glassware for research purposes should be based on levels of contaminants that could potentially interfere with the research requirements. The usual approach is to establish acceptance criteria of visual cleanliness. Additional requirement for ultra clean glassware can be handled on an individual basis using special cleaning processes (over and above the routine glassware procedure) to meet those needs. For cGMP application, acceptance criteria should be scientifically established and residues must be quantified using validated analytical methods. The cleaning agent supplier may provide analytical methods for determining detergent residues by rinse or swab sampling using HPLC, conductivity, or TOC, among others.

A2: We had used one simple method for checking soap solution residues in cleaned and rinsed glassware.

The Soap solutions are alkali in nature. So one can identify the residues by adding universal PH indicator in one of the the cleaned glassware. PPb level of residues shall be identified by HPLC method.

A3: Might be true if one used soap per se - metallic salt of a fatty acid - and its water solution from the actual cleaning operation is alkaline, esp. considering the nature of the soil and the rinse water pH. One could have fatty acids salts (esp. calcium) and I'm not sure that would give an obvious pH reaction.

I assume that many use detergents/surfactants rather than "soap."

In any case, you'd need to validate the procedure.

A4: Yes, non foaming solution for the glassware cleaning is available in the market mainly suitable for pharma. During the glassware cleaning validation ,procedure in place needs to be defined to ensure the cleaning with the predefined steps of cleaning, and the acceptance criteria ensuring to achieve in the same.

A5: There are many commercially available soap solutions for glassware washing. What is acceptable to your organization may not be acceptable to others because of the lack of documentation and support associated with the formulated detergent. In my opinion, it is best to go with a reputable manufacturer that provides a consistent product that has a formal change notification policy that will provide sufficient notice should a component or a critical attribute in the formulation have to be changed. That way, with sufficient advance notification, your organization will have time to put a plan or critical change in place to chart a path forward to minimize adverse impact to your existing validation and/or products.

The manufacturer should also provide toxicity data for the formulated detergent in order for you to calculate the acceptance criteria for detergent carryover for your product(s) or "Worst Case" product from a dosage perspective. See following calculation procedure for determining your detergent carryover limit:

ADI = LD50 (of detergent) X drug dosage body weight (conversion factor "105")
Limit (ppm)= ADI of cleaning agent X 106 maximum dose of next product

Example Calculation Follows:

Cleaner with oral LD50 of 860 mg/kg such as; (CIP 100) Subsequent product dosed at maximum of 15 grams (or 15,000 mg) per day for a 60 kg person
Conversion factor of 100,000 for ADI purposes (Conservative)
* ADI = 860mg/kg X 60kg = 0.52 mg 100,000
* Limit = 0.52 X 106 = 35 ppm 15,000

(Use this to calculate surface area limit; calculation will often result in surface area limit well above customary visual limit of 4µg/cm2) 35ppm X 25 cm2 / 20 mL (solvent desorption volume for swab) = 43.75 ug/mL

This detergent acceptance limit then could be tested with either a validated TOC or HPLC method.

A6: There is only one item I would add to your formula - the swab recovery percentage. Say you have a recovery of 70%, based on your recovery studies, the final limit would be 43.75 mcg/mL x 0.70 = 30.6 mcg/mL

Efficiency in Making TSB

Currently we make upwards of 40L/week of TSB, this is done in 4L batches which is not the most efficient. I was wondering if anyone is aware of any systems that would help in creating larger volumes at one time.

Use of in-house isolates for media growth promotion

We're all aware of the recent event involving the recall of infant analgesic liquids, and the associated compliance issues at the manufacturing site. In reading through the 483 observations from the April 2010 inspection, I came across an interesting one (Observation #12): "...the firm does not test TSA...during growth promotion tests for microorganisms to include for example, molds, yeasts and other potential KNOWN ENVIRONMENTAL CONTAMINANTS FOUND IN THE MANUFACTURING FACILITY AND/OR RAW MATERIALS". (caps added for emphasis)

I've previously posted feedback requests on the PMF regarding the use of in-house isolates in media GP. The responses were varied, but included an opinion that the use of these organisms is scientifically irrelevant. The reasoning included (a) EM isolates cease to become "environmental" once they've been cultured in the lab (the same could be said for ATCC organisms), and (b) it's too difficult to properly standardize and maintain them. As we all well know, "science" and "regulations" often clash. Given the above passage from the 483 observation, what are your thoughts on this topic? Should we try to argue the scientific irrelevancy of using in-house isolates, and risk receiving a 483, or should we just comply with regulatory expectations?

A1: If you freeze your in-house isolates and regulate the number of passes that can be used for growth promotion it becomes a lot more relevant. I have encountered several situations where the in-house isolate would not pass GP but the ATCC supplied organism of the same genus and species grew just fine.

A2: This is one of the problems with using 483 observations as "cGMP". They are not. They are the observations of one inspector who may or may not be knowledgeable in microbiology. Many inspectors are a bit light in academic training and experience in a particular field (only to be expected) and may be working off their understanding of a checklist rather than a deep understanding of the field.

The company receiving the 483 observation, in turn, makes a business decision as to whether they will lose more money complying with a questionable request, or delaying approval by arguing. Usually the company goes along with the less onerous requests and a new "best practice" is born.

Please do not confuse 483s with cGMP. That path leads to confusion trying to line up all observations by dozens of individual FDA inspectors who do not necessarily issue observations from a unified view of "cGMP". FDA does a great job of training, but it is not possible to turn someone into a lab microbiologist

with a couple weeks of lecture and some OJT before turning them loose as the technical inspector. The inspectors are doing their best, good people all. However, mistakes (errors/misunderstandings/inaccuracies) will happen.

Try to design your lab practice with good science. Be aware of 483 observations but do not let them unduly influence your practice. The best "defense" against a 483 observation is to have a documented, supported (literature or textbook referenced) rationale signed off by your Quality organization.

PS - anyone who thinks the FDA inspectors have an easy job should try inspecting a broad range of pharma companies some time. There are some that will curl your hair!

A3: How did you consider GP successful if in-house isolates failed?

A4: I have not encountered a situation where the in-house frozen environmental isolates failed GP. I guess this is where performing a routine effectiveness check on the in-house frozen stocks should be emphasized. For example if you freeze down 100 vials (0.5 ml) of your environmental isolate and you use these vials (one time use only) for a year, you should freeze thaw 1-2 vials once every 3 or 6 months and perform serial plating (to check cfu) and also perform an ID to confirm the organism type. If any of the above fails you should discard your frozen isolates for that organism and start over. This seems like a lot of work but definitely saves us the time and money that goes into investigating a GP failure.

A5: To comply with the testing of environmental isolates, we use new isolates from ongoing tests that have just been identified in recent tests. The isolate had to be a gram negative and have >80 % identification to the species level. For example, for the R2A and PCA growth promotion we would test with a recent isolate from our water test.

There was no need to keep the isolates as part of our stock cultures, just our weekly working culture.

I hope this strategy works out with your lab, we never had problems and complied with the regulatory expectations.

A6: I have just read [name redacted] reply. I agree with and appreciate his response. Each time I review our Growth Promotion SOP I enter into this same debate. Yes, we have set rules on enrolling isolates from the EM Program into our Growth Promotion panel, but I question the bacteriological merit of this practice.

Much in the same way that potable water is assessed by using a class of microorganisms (coliforms) to indicate contact with enteric pathogens, our compendial growth promotion requirements use a selection of microorganisms from which favorable recovery indicates suitability for use in the culture of samples for sterility and environmental monitoring. We agree that these growth promotion tests only augment and not replace the requirement that culture media be used from approved vendors demonstrating sound quality systems.

Acceptable user compendial growth promotion tests are indicators that the culture media will recover a broad spectrum of microbial isolates. What then is the value add of the performance of a Staphylococcus or Bacillus species (for example) once recovered from our environment and propagated for release testing of culture media to our growth promotion programs?

We are responsible for managing myths and perceptions with sound science and I look forward to the clarity and logic that our consensus could bring to this topic. This increases our self confidence and ability to appropriately address investigators' observations.

A7: As others have said, what you've got is an OBSERVATION- one inspector's opinion. This does not make it a "Regulatory Expectation". There's something in the arguments you list, about taking 'natural' bugs and "un-naturalizing" them by preserving them- but there's also something to be said for having some data to support that indigenous, possibly damaged or stressed organisms in your facility could be

detected by your testing methods (never mind that, if you've got them to preserve, obviously they WERE recovered).

Personally, I don't think this point is a 'show-stopper' as a 483 observation- and many companies simply don't have the qualified equipment and personnel to preserve and maintain stock cultures of their environmental bugs. It's quite a different matter from using a commercially-available, ready-to-use preparation of an ATCC organism. It would be very good data to have, and it would also be useful to use these organisms in disinfectant/sanitizer evaluations as well- but it's not a REQUIREMENT that I'm aware of. It's a "Nice-To-Have". If you've got a -70C freezer and someone with the time and knowledge to freeze down your EM isolates, great- but if not, I don't believe this can be considered a "non-compliance" type of observation.

A8: Nothing will curl my hair as it is long gone! Furthermore I audited regional blood banks in the late 80s and found shocking practices in a small number of these centers

Yes pharmaceutical microbiologists in many companies are attempting to make sense of this 483 observation.

If you designate a particular microorganism as objectionable in a specific dosage form, e.g. B. cepacia in an inhalation product the organism should be isolated

using the microbial method used to test the product. The method qualification requirements for the microbial enumeration tests and screening for the absence of specified

microorganisms are defined by the official compendial tests and does not include a range of objectionable organisms as challenge organisms. That would be impractical.

Microbiologist should be aware of the limitations of the compendial tests. B cepacia is recoverable in the total aerobic microbial count, enrichment with soybean-casein digest broth and the absence of bile-tolerant gram-negative bacteria test but not the tests for the absence of E. coli, S. aureus and P. aeruginosa. For example, enrichment in MacConkey's broth at 42-44 degree c will not select for B. cepacia and the growth of most strains of B. cepacia are inhibited by cetrimide.

Again the growth-promotion test requirements are defined by the compendia and are adequate for the purposes they serve.

In general there is no consensus on the objectionable organisms for a particular dosage form. Is B. cepacia objectionable in an oral suspension used to treat children?

For an organism to be objectionable it should have a clinical history of infection with the root of administration of the product or have the ability to grow in the product affecting the physical and chemical attributes of the product. A multi-use oral suspension should be adequately preserved.

A9: I would argue the science and the logic behind for not trying to recover EM organisms during Growth Promotion test. I totally agree that EM organism will stop to behave as a such after a few transfers and will be nothing more then the purchased one.

Contamination observed in purchased media

I recently came across a media contamination issue where 1/10 plates of Blood agar (purchased) was found to be contaminated with 7-8 different colony types (bacteria + yeast mixed). Since this same batch of media was used in product release testing at my end, I had to generate an investigation which will require some data mining and extensive work to determine the root cause. My preliminary thoughts are that since the contamination was seen on unopened, gamma irradiated sealed plates stored properly per our procedures(all other media plates also stored at that same temp did not show any signs contamination) may be inherent in the media and may have come from the vendor site. Is there an

acceptable failure rate for this purchased media- for example: 1 out of 10 plates from a batch of 10,000 are allowed some contamination, again since these media plates are not claimed as sterile instead are gamma irradiated? If there is such an acceptable failure rate where would I find the appropriate documentation for the same? I am also interested to get the rationale or reasoning as to how did one get to this acceptable failure rate if there is one out there. Ideally every manufacturer should aim for zero contamination but that would be possible in a perfect world right!!

A1: If this is purchased media I would recommend contacting your supplier. They usually want to know about contamination issues and know what their contamination rate is. Also, they will work with you on this.

A2: I did not see any information about the viability of the contaminants you found on the irradiated media. If the contaminants are viable then the contamination may have happened after the product was irradiated. If the contaminants are not viable then the contamination may have happened before the media was irradiated and the irradiation killed the growth. Normally, irradiated media is aseptically poured and tested for sterility prior to irradiation. In other words you have to make sure the media is sterile before sending the finished product for irradiation. Manufacturers usually have a validation for the SAL level of the irradiated product based on bioburden and the irradiation dose range levels. One plate contaminated would indicate that there was a random contamination event somewhere in the process either before or after irradiation. If the batch had a sterility problem during the autoclaving or sterilizing process more plates would be contaminated and not just one or two. Random contamination is hard to screen out once they are in the package because most irradiated plates are in multiple layers of wrapping (i.e. triple wrapped). Usually, contamination testing is done by statistical sampling (e.g. ANSI Sampling Tables) of the batch size and not by checking every unit of the batch for sterility.
The manufacturer should always be informed about these issues so that they can investigate the batch records for any problems and it may turn out that it is within their validated process and specifications. The manufacturer can also issue the customer a written investigation summary to be included with your own investigation records.

Objectionable Organisms Recovered During EM of a Sterile Manufacturing Facility

If gram negative bacteria, mycotoxin producing fungi, Bacillus anthracis Bacillus cereus, Staph aureus or other objectionable microorganisms, are recovered during Environmental Monitoring of a Sterile Manufacturing facility, should they be handled differently than the common environmental and skin flora in terms of investigation, and their impact on the quality of the finished product? Should there be a different procedure in place to evaluate the significance of these "objectionable" organisms in a sterile manufacturing environment?

A1: As you are aware the concept of objectionable organisms as defined in the FDA GMPs applies to non-sterile drug products.

Any organisms isolated in a critical aseptic processing area are a concern.

If you isolate B. anthracis in your manufacturing areas you have other problems!

A2: I have seen the practice of assigning levels to these types of investigations. It depends on qualitative and /or quantitative environmental results in the sampled area and how it relates to product release. This level is determined during the preliminary investigation phase and will determine the reporting system used and who performs the investigation (QC or Manufacturing)

A sterile facility with such detections requires a detailed investigation intended to discover the root cause corrective actions and product impact. Isolation of these microbes indicate that the control systems were not adequate either because they were disrupted or stopped being effective.

A3: The possibility of getting the listed organisms in a sterile area EM will result a immense impact in terms of the CAPA and Risk assessment ,and the disinfectant efficacy study ,if suppose observations are made during the routine manufacturing stages.

Pyrogen Test of Flucloxacilin Sodium sterile

This is the second time I raised this question again regarding Bacterial Endotoxin Test and Pyrogen Test of Flucloxacillin Sodium sterile. In BP it is clearly stated that Pyrogen Test should be performed for Raw Material of Flucloxacillin Sodium sterile but for finished product, Bacterial Endotoxin Test is specified, Endotoxin Limit is not more than 3.5 EU/ml- 9 mg per ml. For Dry vial only powder fill directly under LAF to small vial. There are no addition excipients for that product. So why two different parameters for same materials.

A1: I suggest that this issue needs to be taken up with the BP Commission secretariat. It would seem inappropriate to require both the LAL and the pyrogen test to be applied to these materials.

A2: I think here is the issue of vial endotoxin (LPS of gram negative bacteria, if present after depyrogenation and sterilization by dry heat sterilizer).

A3: I recently asked to BP Secretariat regarding BET and Pyrogen test of Sterile Flucloxacillin sodium. They replied me as below:

Unfortunately I only have a limited amount of information regarding the rationale for the inclusion of the Bacterial endotoxins test in the monograph for Flucloxacillin Injection available to me. The more detailed information relating directly to this has been archived and currently cannot be located.

The current policy of both the European and British Pharmacopoeias is to replace the test for pyrogens, wherever possible, with the test for bacterial endotoxins. It appears from consultation of the available files and minutes that a limit for bacterial endotoxins was proposed by the EP Secretariat for the parent monograph and this was noted and approved for inclusion in the BP formulation monograph. However, the proposed limit has not been adopted for inclusion in the European Pharmacopoeia, presumably because of an absence of data. Without the archived file I am unable to tell if we included the Bacterial endotoxin test based on data we had received or under the presumption that the EP would be revising the monograph.

The British Pharmacopoeia does not preclude manufacturers from employing their own methods in the routine testing of substances and products, although in the event of doubt or dispute the pharmacopoeial method given in the monograph alone is authoritative, as stated in the General Notices Part II and Part III.

I hope that this response has been helpful and apologise that at the moment I am unable to give further details regarding the inclusion of the Bacterial endotoxins test in the monograph for Flucloxacillin Injection. If we are able to locate further information I will forward it to you.

Query - dibasic hydrogen phosphate

Can any one tell me that dibasic hydrogen phosphate in Soyabean Casein Digest Broth (as per USP32)is similar to dipotassium hydrogen phosphate in Soybean Casein Digest Broth (as per EP 6.3)?

A1`: Both are same.

A2: As you mentioned in our mail "Dibasic hydrogen phosphate" has to be corrected as "Dibasic potassium phosphate".

"Dibasic potassium phosphate USP" and "Dipotassium Hydrogen Phosphate EP" are same.

Molecular formula: K_2HPO_4
Molecular weight: 174.2

Plate Observation

One of our contract lab interpreted colony like particles on the plates (Total Aerobic Microbial Count) as colonies and reported results as TNTC. After investigation, it was concluded that the particles from sample formulation (soft gelatin capsules) were the culprits. These particles appeared on the plates depending upon temperature at which sample was dissolved (<45C) in Tryptic Soy Broth.

To avoid unnecessary OOS/TNTC situations, observing the plates before incubation should be one of the Best Microbiology Laboratory Practices.

PET of Lozenges

We are in the manufacturing of Lozenges, from syrupy base with large quantity of aqueous/water missible base. Is PET applicable?

A1: It is my understanding that these types of products usually contain a high level of sugar. Did you perform any water activity level determinations of the lozenge or the liquid base? If the water activity level of the lozenge or sugary base is below 0.6, I see no reason as to why challenge testing would need to be conducted. From my perspective, I would think that preservative effectiveness testing of the solid lozenge would not be applicable if there is a low water activity level.

What type of challenge test method would you use to conduct the analysis? The USP or European Pharmacopeia challenge test methods would not be applicable for determining the preservative adequacy of a solid lozenge. The CTFA has a challenge test method (M-6: A Method for Preservation Testing of Atypical Personal Care Products), that you might be able to use by doing a surface inoculation of lozenges. I would only do this type of challenge testing for informational purposes. For the liquid sugary base, you might want to perform challenge testing to determine how long that it can be stored before it is used to make solid lozenges. I suspect that there is some type of heating step to make the lozenge.

A2: Lozenges like liquid-filled capsule have interiors of low water activity, e.g. 0.2-0.3 hence are not susceptible to microbial growth.

Fo a list of the water activity of many OTC and pharmaceutical drug products I would refer you to the appendix of the book:

Cundell, A. M and A Fontana (Editors) Water Activity Application in the Pharmaceutical Industry Davis Horwood/PDA 2009

Disposal of Positive Blood Cultures and Venting Units

My laboratory uses the BacT/Alert system for blood cultures. We use a venting unit by BD to subculture positive bottles. The venting unit does not allow a safe way to remove it from the bottle without risk of needlestick, so we leave them in the bottle for disposal. Does anyone have a better idea for disposal, or suggestion for a better venting unit?

Enrofloxacin solubility

I was wondering if anyone knows of an ideal way to dissolve enrofloxacin powder (>98% HPLC)? Have found some information on the solubility but I thought I would check with this almighty forum for some ideas as well :)

A1: Floxacins (the whole group) are difficult to dissolve in water (I think you only want to know the water solubility). Floxacins have amphoteric and zwitterionic properties that make them lipid soluble. Because these propeties, Floxacins has 2 pKa values. For the carboxylgroup +-6.0 and the nitrogen of the piperazine group +-8. Between these pH range, the water solubility of floxacins are reduced.

The solubility increasing sharply with decreasing pH below 5 or with increasing pH above +- 10 (counts for all floxacins).
(Reference: norfloxacin Analytical Profiles of drug substances volume 20 Edited by Klaus Florey)

So if you want to make enrofloxacin more water soluble, you have to increase or decrease the pH value.

Here some nice internet references:
http://www.baytril.com/index.php/fuseaction/download/lrn_file/kap2.pdf

http://books.google.nl/books?id=2CUbc0RXvb8C&pg=PA899&lpg=PA899&dq=pKA+enrofloxacin&source=bl&ots=jM9Fz4GunP&sig=WAGQlU2jl2zwmqgZd84pqVIeZqg&hl=nl&ei=-T1QTPuqEoibOMSMqaMB&sa=X&oi=book_result&ct=result&resnum=5&ved=0CDAQ6AEwBA#v=onepage&q=pKA%20enrofloxacin&f=false (Veterinary pharmacology and therapeutics, Volume 2000 page 899)

A2: Generally enrofloxacin liquid preparations for vet are dissolved in KOH.

BIs - Wash or Kill

I would be grateful to receive the opinions of any members actively involved in BI Preparation and usage for validation of wet / spray Peracetic Acid Decontamination systems (in this case an automated SIP system). Do members consider use of inoculated coupons with a wet(spray) systems to be valid. In a continuously drained system, is it necessary to make a distinction between absence of recovery due to wash off of spores and actual kill or is it irrelevant?

Further background details are provided in my previous e-mail which unfortunately didn't elicit any response. In a case where recovery was unlikely I would assume that lab based kill studies followed by actual verification (preferably chemical testing for reduced workload) of dispersal and effective surface coverage would be a more realistic test scenario. Has anyone followed such an approach?

identification of protozoan in water sample

Dear all I need some information regarding identifying following protozoan samples in water ,

Ø Giardia lamblia,

Ø Cryptosporidium spp.,

Ø Cyclosporia spp.

Ø Balantidium coli

A1: You can refer IS standards for microbiological testing of water provided by bureau of Indian standards.

TAPC

We are running USP<61> on one of our products by the plate method (It is not a sterile product). Our laboratory is not staffed on week ends, and we ran several validation studies to confirm growth did not

differ by significant amounts by allowing a 70 hr incubation. Or so we thought. We are currently seeing colonies on our plates from one of our concentrates, but only after the 3rd day. We are in the process of identifying the organism, but...
Has anyone else had this experience? If the growth does not increase the counts over the product specification would you only record results after 48 hrs? wait for further growth? count the APC at 48 hrs, re-incubate the plates and report additional growth if found?

Technically correct and product safety/risk assessments are not always the same.

A1: We always take into account the results once the incubation period is over. That would be the Monday count. And that's the result we report in the CoAs. If the count were over the specs I think we would perform the oos investigation.

A2: Generation time vary with organisms
The appearance of visible growth varies with what phase the organisms are in, stressed etc , the source of product- if there is some inhibitory substance present.
 By providing proper media/ temperature and incubation time we resuscitate them and so some will grow later on.
So I would definitely consider counting after 72 in this kind of situation. You can count after 5 days also, once the colony has grown it will not go away. It might spread or start dieing depending n the type of organism. So if you need for ID you might want to transfer for a fresh isolate when they are alive.

A3: The plates should be read according the incubation period that you have assigned during your validation studies. If the results are satisfactory within that period there is no need for further incubation, even if there is no growth after the incubation period is complete. For a correct risk assessment, sufficient retrospective data should be available to analyze the trend if early release is required due to some urgency, otherwise there could be a quality and regulatory risk. I hope I got your question correctly!

A4: Don't think you can play Wizard of Oz on this - prob better to address the data observed rather than ignore observations as they fall outside the incubation period. As Geldreich showed in his R2A paper, counts go up over time as different populations grow.

A5: Did you ever *ID the organism(s)? Just curious.

A6: We did. The results came back as Paenibacillus lautus. There's not much information out there on this organism, however, the organism does have a tendency to spread across the agar surface with microcolonies. Growth rate is 1-2 mm colonies in 2 to 3 days at 30C.

I'm not quite sure what we are going to do with this information, as this product is sold for food use only.

Does any one have any further information on this organism?

A7: During HVAC qualification we have study and find out worst case location in clean room for viable and non viable count.
Now we are monitoring only one worst case location in routine EM monitoring for viable and non viable count both.
Is it consider one worst case sampling point in routine monitoring? Can you provide us guidelines for the same.

A8: Generally there will be more than one location monitored in an EM program. The number of sites and locations are determined in the qualification study, but should incorporate some redundancy in areas to ensure adequate coverage. Worst case locations are determined by the data.

Some resources that might be helpful:

Conference Oct 4-5 : http://www.microbiologyforum.org/2010/HPA1008/ will look at EM qualification as well as RMM, equipment, water, etc

New book: Laboratory Design: Establishing the Facility and Management Structure https://store.pda.org/bookstore/ProductDetails.aspx?productabbreviation=17294 has a good discussion of the EM program as well as virtually all other aspects of the Micro lab from a facilities and organizational perspective.

Finally a white paper that might be helpful - http://microbiol.microbiologynetwork.net/resources/monographswhite-papers/qualification-of-an-environmental-monitoring-program-1-selectionjustification-of-sample-sites/

Penicillinase activity

I have a product that contains penicillins, so I have to use the penicillinase enzyme in order to inactivate the antibacterial activity during microbiological purity examinations.

The problem is that, according to the customer, 1 ml 8 lac IU of penase has to be added to the sample preparation.

What does "8 lac IU" mean? Please be so kind to explain how is that unit related to "IU" (international unit) or "LU" (levy unit).

A1: Can the customer not translate their own specifications? I would hate to advise you on this topic, only for you to find out from the customer that my interpretation was incorrect (causing you and your lab to waste valuable time and resources).

A2: Your question of 8 lac IU will be 800,000 IU is supposed to be8 Lakhs or Lacs the Indian system used decades ago.

A3: In regarding the activity of penicillinase enzyme first and foremost you have to calculate or convert your qty of penicillin sample present in the sample testing and convert this qty of pencilline to IU or LU or KV unit , i think one LU= 0.8mg of penicillin and on the basis of activity of penicillinase enzyme you can addd that much in the sample that will inactivate the activity of penicillin

A4: I believe that Lac IU may refer to Beta-lactamase activity, a frequent measure of penicillinase activity- but a word of caution: Most Beta-lac manufacturers give activity figures, for at least two types, and these are often ALMOST as accurate as cost estimates originating from the US Government. Develop and qualify an assay for your prepared media to determine that whatever you calculate and use, it WORKS in your plated media- you'll NEED that information, or you'll need lots of headache remedies.

A5: 1LU = 59.3 IU
now we require 800000 IU so, cross multiplication
800000 / 59.3 = 13490 LU
in market we are having penicillinase enzyme with 20000 LU
now prepare this solution as 13490 LU
for example if i want 10 ml of 13490 LU solution from 20000 LU
then C1V1 = C2V2

20000 x V1 = 13490 x 10
V1 = 13490 x 10 / 20000
V1 = 6.7 ml

so now take 6.7 ml of 20000LU penicillinase solution and dilute it in 3.3 ml
of sterile purified water
to make volume of 10 ml.
that 10 ml volume is of 13490 LU concentration
i.e. 800000 IU concentration

now take 1 ml of this solution and proceed for sample preparation.

A6: 1IU will hydrolyze 1.0 micromole of benzylpenicillin per min at pH 7.0 at 25 deg cel, and 450.000 LU/mg powder is equivalent to 750 IU/mg powder.

Media for detection and enumeration of Bifidobacteria and Lactobacillus

Anybody can tell what media is use to detect and enumerate

1/ Bifidobacteria spp only
2/ Lactobacillus spp only
from product containg mixed culture, i.e. Bifido, Lactobacillus acidophillus, Lactobacillus casei, Lactobacillus delbruekii, Streptococcus thermophilus & Lactococcus spp?

A1: I am familiar with L. acidophilus and L. delbruekii from my experience in the yogurt manufacturing industry. S. thermophilus can be isolated on M17 Agar (pH 6.9) @ 42C. L. delbrueckii ssp. Bulgaricus can be isolated on MRS Agar (pH 6.2) @ 42C. All plates should be incubated for 3 days in microaerophilic conditions.

A2: lactobacillus isolation media is available with himedia

A3: For a mixture of Lacto and bifido

- Sample dilution= Prepare in .1% peptone using stomacher and not in phosphate buffer as it can damage lactic acid bacteria .
Prepare necessary dilution -6, -7 -8 or-9
plates are done in triplicates for the required dilution
Rinse the pipets with .1% peptone before using for preparation of dilution and transfers.

-Perform count on MRS agar supplemented with .05% cysteine-HCL(This is the total count)

-Also perform plate count on APT agar (SAMPLE DILUTION IS SAME AS ABOVE)This is Lactobacillus count

Bifidobacter count = Total count- lactobacillus count
Incubation is anaerobic

A4: Bifidobacteria spp on BSM Media
Lactobacillus spp on MRS Media

A5: Can anybody tell me that why the confirmatory test for specified organisms in USP 32 chapter <62> and EP 6.0,6.3,6.7 are not specified clearly. I feel that if both the pharmacopeia are harmonised than it should have same methods but it is not clear.

A6: I think you are from Sterling gelatin plant. First, witch method you are not clear? Hope you display the method.

Microbiological assay by turbidimetric method

We are planning to initiate microbiology assay by turbidimetric method. Sample name is Thiostrepton as per USP 32 <81>. According to USP , final standard stock concentration is 1 U and median concentration is 0.8 U.How to prepare the S1,S2,S3,S4 and S5 with 1:1.25 ratio. Can any one help in this regard?

Frequency of use for sporicidal disinfectant

Our company is about to start using a sporicidal disinfectant. We are struggling with finding a frequency of use for this product. We have heard recommendations from weekly to monthly, to every two months.

We would like to take a science based approach to implementing this at approximately 60 different locations across the US. Can anyone point me to some literature that has defined experiments that we may be able to perform in order to validate the periodicity of use for a sporicidal disinfectant?

A1: I would base the rotation on the environmental monitoring data for each facility. If there is evidence of spore forming fungi/bacteria, then use the sporicide as part of your cleaning program on a rotation that keeps the population under control based on the data. I'm thinking the variation on use cycle is based on exactly these kinds of findings at different facilities.

A2: To make this decision you really need to look at your environmental monitoring data. Are you recovering spore-forming organisms frequently? Are you exceeding alert and/or action limits? If so, then you will want to use a sporicidal agent more frequently (weekly). If not then you could use it less often. Let the data tell you the frequency that makes sense for your facility.

A3: I don't think there is one good answer for this. Many companies utilize a monthly regimen. But this is going to depend on the type of facility (non-sterile vs aseptic processing), which area you are speaking of (i.e. which airborne cleanliness classification), etc. Ultimately an extensive PQ validation over the course of perhaps a year which shed some light as to the frequency to keep spore forming organisms below a certain level you have deemed "acceptable" and allowing for seasonal variations. I think your data may suggest there is increased frequency at certain times of the year perhaps. The other conundrum is your desire to apply the same frequency across 50 sites. I think each site is it's own entity, and while ultimately you may find that the same frequency or general procedures apply across the board, I feel you may be misguided to not consider facility to facility variations based up many factors including geographic variation, product/process differences, gowning and personnel habit differences, etc. Each facility will probably become its own microcosm, its own unqiue environment that behaves differently with respect to its surround similar to high purity water systems.

A4: The frequency for the application of a sporicide should be based on each facilities historical EM surface monitoring data in relation to the distribution of spore-forming microbes.

A5: I generally recommend the use of a sporicide based upon your Environmental monitoring data, isolate identification and data trending analysis. So, frequency should be based on the common occurrences of fungal and bacterial spores. Most pharmaceutical, biotech, and medical device sites will have a frequency set at once a week, once a month, or once a quarter based upon the environmental trending. This frequency may increase as needed during excursions, construction, extraordinary cleaning events, and warm and humid summer days. Please feel free to contact me directly for additional information. Here are three helpful references:

Bartnett, C. Polarine, J. and Lopolito, P. (2007) Control Strategies for Fungal Contamination in Cleanrooms Controlled Environments September 2007

Polarine, J., Karnaja, P., Macauley, J., and Klein, D. (2009) The Activity of Disinfectants Against Common Cleanroom Fungi Cleanrooms Magazine February 2009.

Martinez, JE The rotation of Disinfectant Principles True or False Pharmaceutical Technology (2009)

A6: I fully agree with forum members on establishing routine sporicide frequency of use. You should also consider not only routine frequency but also non-routine usage. These products are often employed: In the event of a breach (in cleanroom) After planned maintenance.

At first sight of unusual or objectionale organisms (outside of the routine orgs picked up in environmental monitoring eg skin borne G+ve's) Manufactruing failure.

Question on PDA Technical Report #1

A question concerning PDA Technical Report #1 "PDA Technical Report 1, Revised 2007, (TR 1) Validation of Moist Heat Sterilization Processes Cycle Design, Development, Qualification and Ongoing Control"

For validation/revalidation of porous loads, is it necessary to spike stoppers with a microbiological indicator if, after doing the D-value determinations, the D-values of the microorganisms on the stoppers are less than the BI strips with Geobacillus stearothermophilus?

Propionibacterium acnes - Culture Maintenance

I want to know in which media P.acnes is preserved long time (for 1 month at 2 to 4 °C). As this organism is microaerophillic should I overlay a layer of liquid paraffin on the grown broth media to maintain the anaerobic conditions. We are about to perform MIC of some essential oils on P.acnes should I opt for Macrobroth dilution assay or agar dilution assay, which method would be better for anaerobes. Please suggest me proper media and method for preservation of pure culture and MIC.

Expiration date for cryopreserved isolates

I have a question regarding expiration dating of cryopreserved isolates. Our lab has a practice of reviving a lyophilized ATCC vial, streaking several plates and cryo preserving a number of vials in TSB broth with 15% glycerol. These vials are then stored at -80C. Do these vials need an expiration date? If so, what should it be?

A1: Your probably need to validate expiry date for the isolates that you are working on so as not loose a "precious" strain if you have one. But generally one is able to revive most isolates after over a decade.

about an antibiotic susceptibility culture media for fungi

I would like to know that what is the best culture media for antibiotic sensitive test for fungi? Hope to get reply from experts.

A1: To know the action of antibiotic I would like to give you a brief insight of its mode of action which may help you to understand the selection of media for fungi. So, Antibiotics active against bacteria are bacteriostatic or bactericidal; that is, they either inhibit growth of susceptible organisms or destroy them. On the basis of their mechanism of action, antibiotics are classified as (1) those that affect bacterial cell-wall biosynthesis, causing loss of viability and often cell lysis (penicillins and cephalosporins, bacitracin, cycloserine, vancomycin); (2) those that act directly on the cell membrane, affecting its barrier function and leading to leakage of intracellular components (polymyxin); (3) those that interfere with protein biosynthesis (chloramphenicol, tetracyclines, erythromycin, spectinomycin, streptomycin, gentamicin); (4) those that affect nucleic acid biosynthesis (rifampicin, novobiocin, quinolones); and (5) those that block specific steps in intermediary metabolism (sulfonamides, trimethoprim).

Antibiotics active against fungi are fungistatic or fungicidal. Their mechanisms of action include (1) interaction with the cell membrane, leading to leakage of cytoplasmic components (amphotericin, nystatin); (2) interference with the synthesis of membrane components (ketoconazole, fluconazole); (3) interference with nucleic acid synthesis (5-fluorocytosine); and (4) interference with microtubule assembly (griseofulvin).

For an antibiotic to be effective, it must first reach the target site of action on or in the microbial cell. It must also reach the body site at which the infective microorganism resides in sufficient concentration, and remain there long enough to exert its effect. The concentration in the body must remain below that

which is toxic to the human cells. The effectiveness of an antibiotic also depends on the severity of the infection and the immune system of the body, being significantly reduced when the immune system is impaired.
You must know the chemicals sensitive to fungi are also clinically useful antibiotics which include amphotericin B, nystatin, griseofulvin and the azole antifungals
The best culture media for AST is Mueller-Hinton agar (MHA) which contains beef infusion, peptone, and starch and is used primarily for antibiotic susceptibility testing. It can be in a form of blood agar.
Some other Fungal media of interest is
Sabouraud agar: Sabouraud agar is used to culture fungi and has a low pH that inhibits the growth of most bacteria; it also contains the antibiotic gentamicin to specifically inhibit the growth of Gram-negative bacteria.
Hay infusion agar: Specific for the culturing of slime moulds (which are not fungi).
Potato dextrose agar : PDA is used to culture certain types of fungi.
Malt extract agar : Malt extract agar has a high content of peptone and is acidic. It is essentially used in the isolation of fungal microorganisms.

A2: You can Use RPMI-1640 media for Microbroth dilution antifungal susceptibility test, Refer CLSI standard.

rapid method

There´s somebody works with BioLumix Scientific or other rapid method?
What is your opinion?

A1: Have been product manager for rapid micro solutions, mostly bioburden testing, so I do have some knowledge on the area, but BioLumix is a but limited and I have a very different position now in another company -which applications are you looking for?

A2: The Biolumix technology is based on monitoring changes in a broth medium with unique dyes in which target microorganisms grow and are detected by changes in color or fluorescence as the result of the production of carbon dioxide. A variety of tests are available including total viable count (or total aerobic count), coliform, E. coli, the combined testing of coliform and E. coli in a single vial, yeast & mold, lactic acid bacteria, Enterobacteriaceae, Salmonella, Pseudomonas, Staphylococcus, Microbial Limits, probiotics, sanitation monitoring, shelf-life predictions, challenge tests, environmental monitoring, enzymatic reactions, fermentation process, growth kinetics for mathematical modeling, effects of inhibitory systems and growth parameters (pH, temperature, nutrients) on growth kinetics, and spoilage flora. I have a nice overview of this system including pictures on my website. The web address is: http://rapidmicromethods.com/technologies/technologies_biolumix.html.

As for the second part of your question ("opinions of other rapid methods"), this is rather broad as there are dozens of RMM technologies for a variety of applications and test samples. Can you be a bit more specific on what your needs are?

Alternative to bleach

I was wondering if anyone could recommend an alternative sanitizing agent that they currently use in their facility other than bleach for use on stainless steel equipment. I have found many good sanitizing agents but many of them pose a large safety risk or take too long.

A1: What is the application for your sanitizer? What temperature is this product being utilized at? Room temp? For instance, bleach is less effective above 50 C as the chlorine is rapidly lost to the atmosphere. Is this in an industrial setting such as for food contact surfaces? Although bleach is a good sterilizing agent, it is also corrosive (especially to stainless steel). Hopefully you currently rinse off any residual bleach. 70% isopropyl alcohol (although more expensive than bleach) might be a good alternative.

Please provide more information.

A2: We use CIP100 and CIP300 to soak the small parts and manually scrub larger items (you will likely need to neutralize the CIP before dumping down the drain). For wiping down the outside of stainless equipment as a general room cleaning we use Vesphene and LpHSe. Rarely do we use Spor-Klenz (once monthly with additional applications when an increase in spore-formers is noted).
You could also use Hypochlor - though this is just bleach diluted with hydrogen peroxide, so you would have the same rusting issues.

A3: There are a few alternatives to bleach Most sites use a products like bleach (oxidising agents) as a sporicide you don't state the target organisms. Products with similar spectrum and speed to bleach are: blends of hydrogen peroxide and peracetic acid peracetic acid chlorine dioxide

They all have their pro's and con's but the prime considerations will be The grade of stainless steel you wish to disinfect (though bleach is probably the most corrosive on stainless) the in-use concentration and contact time how you buy the product (pre-diluted or concentrate) manual handling H&S dosing method

In my view overall, these products are no more hazardous than bleach but they may have different hazard profiles to bleach.

A4: We use sporklenz ready to use, produced by Steris as a sporicide. We also use vesphene and Lph, also produced by Steris for vegetative cells.

A5: Over in the UK we currently use "biocides A and B" that are produced by Shield Medicare: http://www.shieldmedicare.com/. I think they are now selling this internationally so it may be of some use to you.

A6: Viriditec is a highly effective sanitizer which releases the natural oxidizing power of activated oxygen.

A7: There are many options as far as sporicidal agents such as hydrogen peroxide/peracetic acid blends, hydrogen peroxide, chlorine dioxide, and aldehydes. If your objective is to address vegetative bacteria then phenolics or quaternary ammonium compounds are possible choices. There are pros and cons to all of these products. Hydrogen peroxide/peracetic acid blends will provide sporicidal activity with less hard surface corrosiveness versus Bleach at highly effective concentrations or versus acidified Bleach.   Feel free to contact me directly if you would like some information on antimicrobial activity of various products and the compatibility of some of these products with common cleanroom substrates.

Validation of Swabs

I am planing to validate new swabs for our environmental monitoring. Has anyone performed any studies on this? Since the swabs will be placed in the TSB (Tryptic Soy Broth), do I need to do recovery studies for them or growth no growth is good enough?

A1: I would recommend doing experiments to validate the new swabs recovery and release capacity against your traditional swabs. I would define recovery as the ability of the swab to collect and retrieve viable microorganisms from a surface. The release capacity is the ability of the swab to release any collected sample into a solution or media. Use Pharmacopoeial strains such as S. aureus, P. aeruginosa, E. coli, B. subtilis, C. albicans, A. niger and environmentally isolated strains.

A2: The sad fact is, of all the methods routinely used for environmental monitoring, swabs probably easily take the prize for WORST recovery, quantitatively speaking - they are really inefficient, besides being somewhat difficult to develop credible validation methods for. I've tried a number of studies on swabs, and I tell you honestly that if I got 3-4% recovery, numerically, I thought it was a very good day indeed. Consequently, I suggest that quantitative recovery studies on swabs fall into the category of 'questions you shouldn't ask because you know you won't like the answers'.

Qualitative studies, using swabs to recover SOME growth from surfaces contaminated with low numbers of microorganisms, might be your best approach, if you have areas (fixed equipment, narrow spaces, etc.) where you cannot use RODAC plates or other, better surface-sampling methods. These can be recorded as growth/no growth, and shouldn't be too difficult to perform- although simple drying or desiccation of many organisms after inoculation onto surfaces, is enough to reduce their numbers to levels undetectable by swabs. Direct inoculation into TSB is probably your best bet- any kind of transport of the swabs between sampling and inoculation, any additional time delay at all, will further reduce already-poor odds of recovery.

A3: Unless you can show equivalence to your previous swab I think you will have to revalidate, few things that come to mind. Is the swab material consistent batch to batch from you supplier Assume they are sterile when purchased and do not have to be processed in-house The nature of the swab device and does it fundamentally alter how operators take the swab. Is there any change of swabbing method? The material does it have any effect on the organisms picked up How easily are organisms recovered from the swab Are the new swabs equally effective (more effective or even less effective) at picking up viable organisms from your surfaces How long do orgs retain viability on the swab device.

A4: Seconded! I have seen Internet reports suggesting that swabs only pick up 20% of bugs present, and of those, only release 20% onto growth media. I therefore mention in my SOP/ Work Instructions/ specifications, that for swabs used in Clean Room, any positive results are immediately actionable...don't bother with Alert level isolations; Target of No growth.

A5: Another agreement. Quantitative microbial sampling with swabs of any type is just about impossible. Calcium alginate swabs will dissolve completely and release all organisms picked up, but 20% recovery from a surface is about the best you can expect. There are several manufacturers that make kits - a sterile wetted swab in neutralizing buffer and a sterile TSB tube packaged together. We use these for +/- tests in clean areas and they eliminate most of the problem with drying of organisms.

A6: If I can just state, isn't it most critical to use the same method and detect drift from "ideal or normal" operating conditions? So long as the method and technique is the same and well controlled in a procedure, in theory the important part is to detect shift or change. Is this the ultimate intent/goal of a microbiological monitoring program, Yes?

A7: I am also in total agreement with your statements, just wanted to list the variables that cause the recoveries to be so low when we did or validation trials:

1. Pack Density of the swab would retain organisms and prevented us from improving extraction.
2. Analyst swabbing Practice/Technique would affect recoveries among individuals. Examples of variability were swab rotation technique on the sampled surface and amount of pressure during swabbing.
3. Transfer efficiency from the swab to the testing media. Variability in vortexing and differences among microorganisms were some of these factors.
4. Swab holding time.
5. Type of surface under test: Stainless Steel Aluminum and Plastic.

During our validation studies we discovered that the swab could retain from 34 to 90% of the inoculated organism and the surface (stainless steel coupon) could also retain from 5 to 45% of the inoculated organism.

All this variability makes validation an impossible task. In my personal opinion, this surface recovery test is not intended to be quantitative but rather qualitative.

A8: Absolutely agree, and this is another discussion specific to changing boats mid-stream. There are newer, apparently better swabs coming into the market place, and if they really do pick up, and release more bugs than those used during the original set up of the monitoring regime then you're almost certainly shifting the goal posts. There is a major risk that using better testing methods will suddenly cause all the results to be OOS, so you really need to perform a whole new performance qualification of the cleanroom

facility and set new Alert and Action limits...weigh that up against due diligence, and cost effectiveness and you've got a massive event on your hands!

A9: Isn't the ultimate objective to make safe and wholesome products? If current sample acquisition devices are missing events that newer options can detect, isn't it better to switch? No question it is important to "detect drift from ideal or normal operating conditions". However, what if true (and potentially preventable) problems go undetected during production?

A10: I guess the question is really what does EM measure? The assumption in your answer is that more sensitive EM measures (surface sampling techniques) will lead to safer product production. I think this is an unfounded assumption.

The point of EM is a measure of the state of control of your facility, not the microbial quality of the finished product. This critical distinction is described in USP, the FDA aseptic guide, and expanded upon in two recent articles:

Hussong, D and RE Madsen. 2004. Analysis of Environmental Microbiology Data From Cleanroom Samples. Pharm Technol. Aseptic Proc: 10-15

Farrington, JK. 2005. Environmental Monitoring in Pharmaceutical Manufacturing - A Product Risk Issue. Amer Pharm Rev. 8(4):26-30

Therefore, no - what is important is the data trend (assuming the data is of acceptable quality). Changing the measurement method is actually contra-indicated in this instance unless there is a specific issue that must be corrected.

Of course, if we were able to demonstrate that the current method missed true and potentially preventable problems during production this would be a reason to change. Basic GMP requires that we would also need evidence that the new and preferred method ("corrective action") would fix the problem. What might these problems be and how can we show that the new swabs now allow us to see the problems to prevent them?

A11: I need a reference method on the initial validation of sampling swab such as recovery study and etc. Where can I get this?

A12: Please help me I need a specification that I can use for the swabs when taking the micro samples for the cleaning or fractionation tanks.

A13: Swab Test specification is available in USP chapter, Microbiological Evaluation of Cleanroom. Please find that chapter and there everything is clearly mentioned.

A14: We use RO water.

A15: You may find the following reference useful for further information on Nylon-flocked swabs that are now available and effective for quantitative sampling of surfaces during environmental monitoring:

"Qualification of High-Recovery, Flocked Swabs as Compared to Traditional Rayon Swabs for Microbiological Monitoring of Surfaces"
Gilberto Dalmaso, Manuela Bini, Roberto Paroni, Michela Ferrari
PDA J Pharmaceutical Science and Technology. 2008 May-June Vol. 62, No.3, pgs 191-199

A16: What water are you using to clean the vesselsl? RO water? Then use NGT 100 cfu/25 cm2, I have seen lower specs like NGT 10 cfu/25 cm2, these however may prove to be too tight.

Solid Dosage Micro Benchmarking

Just a quick survey for everyone working with solid dosage units (primarily tablets and capsules) in the US.
What kind of microbiology related issues have FDA inspectors been focusing on in 2009 and 2010? Even if they didn't end up on a 483.
Are they looking much at environmental monitoring in the un-controlled areas?
Any specific focus on product testing?
Any expectations for "new" test method validations? Equipment cleaning?
Any other microbiology related hot topics?
I'm helping prepare for an anticipated inspection coming up soon.

A1: For our solid dosage contract manufacturing facilities (OTC) the FDA is looking at complaints, method validation, equipment and process validation and in-process deviations. They always discuss product stability test results at some point in the inspections.

A2: Why the emphasis on stability?

Microbial testing would not be a critical stability attribute. A tablet or capsule stored in a suitable sealed container would not achieve a water activity to support microbial growth. This would be >0.70.

I recommend that you read Linda Showronsky's chapter in the book I co-edited entitled The water activity application in the pharmaceutical industry.

A3: No emphasis on stability here............just that all FDA inspectors look at stability data for solid dosage forms at some point of the inspection. They always look at disintegration, dissolution, assay, etc. attribute data. Water activity is always a good discussion (or education) point with auditors.

USP <231>

I was wondering if anyone has any insight into USP <231> heavy metals as lead. I would like to use ICP to determine heavy metals rather than these long drawn out procedures referenced in the monographs.

A1: ICP especially with MS detection is a completely acceptable method for testing of HM. The use of alternative analytical technology is allowed by both USP and Ph.Eur. Typically, a comparability study is really all that you need to complete to cover any regulatory issues. If you don't want to do anything we (AAIPharma) can actually test your samples by either the USP<231> method or ICP-MS.

A2: USP General Notices addresses the use of alternative procedures to the referee tests found in monographs and general chapters. Here is an excerpt from the pertinent section in GN:

"6.30. Alternative and Harmonized Methods and Procedures Alternative methods and/or procedures may be used if they provide advantages in terms of accuracy, sensitivity, precision, selectivity, or adaptability to automation or computerized data reduction, or in other special circumstances. Such alternative procedures and methods shall be validated as described in the general chapter Validation of Compendial Procedures <1225> and must be shown to give equivalent or better results. Only those results obtained by the methods and procedures given in the compendium are conclusive."

In other words, your alternative ICP method may be used if you can prove that it is equivalent or superior to the methods in <231> and validate it per <1227> for use with your products, which includes assuring there are no matrix interferences.

By the way, these allowances also apply to alternative micro methods such as RMMs.

query on Objectionable Microorganism in Finished Products/API

I would like to have certain queries answered from experts related to "Objectionable Organism" in non sterile products as per the requirements of USP general chapter <1111>- MICROBIOLOGICAL ATTRIBUTES OF NONSTERILE PHARMACEUTICAL PRODUCTS and various regulatory guidelines like USFDA, TGA etc:

* Is the requirements to be fulfilled for finished product only, or to be extended to API, excipients, water, environmental analysis, etc?
* Is the requirements to be fulfilled for all the range of products manufactured in the company?
* Is the requirements of USP general chapter (<1111>) mandatory, since above chapters 1000, they are only to be used as a reference chapters only.
* The risk based approach if used, needs to be a part of organization policy/company manufacturing documents like SOP, General procedure
* If so, what should be the requirements, acceptance criteria, evaluation criteria, and an approach to achieve.
* This should be applicable to all the products been distributed to all the geographic regions, i.e. USA, Europe, less regulated markets etc.
* If any non-specific organisms do arises on the plates been used for specific organism (i.e. E.coli, Ps. aeruginosa, Salmonella sp, and S.aureus) and TAMC/TYMC should it be evaluated till species level by Vitek 2 or API analyzer.
* Should it be a continuous process of testing all the products been manufactured on the daily basis (thereby increasing the time period of release thus hampering the business and work load of the Microbiologists?) or should be done once for all the range of the products been manufactured?
* The concept of performing this kind of activity will eventually surpass the microbial limit test indicator organism or not?

This is related to a query been generated by some competence regulatory authority.

A1: This is a significant topic, and one that is really beyond the scope of a single Email. In short, there is a big difference between the compendial Microbial Limits Tests and the manufacturer's responsibility to put safe products on the marketplace. The compendia do not concern themselves with "objectionables" (except for some information in <1111>) but rather deal with "specified" organisms.

As for finished product tests - your company should be able to document the safety of every batch of non-sterile product released. This is the thrust of the CGMP. I would urge batch-specific testing unless you can absolutely guarantee that every batch is identically contaminated (ie non-sterile) every day.

There is a white paper that might help located here: http://bit.ly/c0Ik1H

Do take a look at the white paper to see if it helps.

A2: A question about a specific Objectionable Organism scenario. Anyone got any thoughts why the FDA Bad Bug Book should be citing under Miscellaneous Enterics, genera such as Klebsiella, Serratia, Citrobacter etc. as "Food Borne" when the model of virulence suggested is one of plasmid transfer of virulence factors from e.g. STEC? For mine, I can't see this transfer happening in a food medium...much more likely to occur in the gut after ingestion?

Furthermore, the examples I can find , which incidentally are not actually referenced in the BBB at all, relate to very small and specific groups of victims who are either HIV positive or sub 3 year old infants... and in India or Africa...and there is no suggestion that these modified species were actually originally ingested in a causative condition, merely that they were isolated from faeces of sick people, and may therefore be assumed to have contributed to the symptoms. How then can the FDA BBB put such a scare into people without fully qualifying the context of the concern?

Antimicrobial Preservative Efficacy Testing

Can anyone help me with a dilemma regarding pass/fail criteria please.

I have been failing tests unless the results were 1,2,3 log or over. During recent audit I have been told, that rounding is allowed in these tests and anything over 0.6, 1.6, 2.6 will comply with requirements. If this is the case where is this documented?

P. acnes - MIC and culturing

I want to know in which media P.acnes is preserved long time (for 1 month at 2 to 4 °C). As this organism is microaerophillic should I overlay a layer of liquid paraffin on the grown broth media to maintain the anaerobic conditions. We are about to perform MIC of some essential oils on P.acnes should I opt for Macrobroth dilution assay or agar dilution assay, which method would be better for anaerobes. Please suggest me proper media and method for preservation of pure culture and MIC.

inactivation of anti-microbial activity

I am working in the company which is manufacturing the sterile cephalosporins API & DP. while doing sterility test our practice is , we will add penase to neutralize the antimicrobial activity of the product. is the neutralization is necessary?

And also during the manufacturing activity when we performing the monitoring activity we are also adding the penase to the monitoring plates that are going to be exposed in the manufacturing area. is this necessary

My question is if we add penase to the monitoring plate when the product comes in contact with the media the activity will be neutralized when the product becomes liquid only ? is the product dissolves when drops on the media plate is that the correct process to use penase.

While performing the sterility test is the penase addition is necessary can any one suggest regarding the same.

A1:
1. Suppose your product is contaminated with cephalosporine sensitive organism, when you do your sterility testing if you have trace amount of the antibiotic the organism will not grow therefore you will not be able to detect it in your incubation. However that does not mean that the organism is dead. This is particularly true when you are dealing with bacteriostatic drugs. When you remove the organism from the hostile environment and put it in a nice happy environment (sick people)... then it will proliferate. It is possible for organisms to stay dormant but viable in bacteriostatic drugs (particularly powders) for months.

2. Regarding agar plates, remember that agar plates are >95% water. Therefore for all practical purposes, macromolecules are in a fluid environment and are freely diffusible.

A2: Are you sure to use penase for cephalosporin, it is penase for penicillin antibiotics. Sure the product with the contact of media will get dissolved and as the media plates is with the neutralizer it will work and retain the mom if any.

Yes during the sterility testing requirement of neutralizer should be with the peptone used for rinsing and should be given validated contact time for the neutralization of antibiotic.

A3: Surely the adding of penase is necessary, but you have to perform tests in order to establish concentrations and to validate your procedures with each one of the cephalosporins.

A4: Surely you have to add the penase and before sterility testing you have to validate your test method with betalactamase and without betalactamase finally you can consider for without betalactamase method for routine testing.

A5: Everyone is replied with correct comments. You raised two questions as below :

1. During Product validation of sterility test, you have to remember one thing that the residual content of antibiotics is neutralized. That may be penase(Penase is only for Penicillin product, Broad spectrum Beta Lactamase Enzyme for Cephalosporins & Penicillin products) or any other neutralizer. So Penase is not only mandatory neutralizer, you can inoculate Meat peptone for rinsing of membrane filter for removing of product residue. Validation will tell which neutralizer need for your product. Beta Lactamase enzyme is very costly, so initially you can try with meat peptone.

2. During environmental monitoring you used also penase. It may be required when the product power circulate in air. Normally the neutralizer is not required into plate for environmental monitoring, only in Class 100 (LAFWS Filling point) product dust may inhibit your growth, so in that case you have to use penase or any others neutralizer into plate before use for environmental monitoring. I have been working for 16 years in dry vial environmental monitoring , the neutralizer has not been used in plate.

Treating waste

Currently we accrue large amounts of liquid waste that could potentially be biohazardous (~40L/week). Currently we put it through an autoclave cycle to sterilize it prior to dumping it down the drain, however this is taking up a lot of our man-hours and resources. I was wondering if there were suggestions as to a more efficient way of dealing with this, including some ideas that have come up:

1. just toss it down the drain. This was proposed and I am not in favour of this, one reason being at the time we dump the contents we have no way of knowing what could potentially be in the solution (most solutions come from absence testing of E.coli/Salmonella/Staph/Pseudomonas).

2. Chemical treatment - what are preferred chemicals used, would 10% bleach solution be acceptable for this?

3. Any other ideas?

A1: You can send the waste to a residue treatment plant.

Air samplers

I am trying to find out about air samplers for EM. Right now we are using the Biotest RCS system, but are thinking about changing. We are not a pharmaceutical company and am not sure there is reason to change. Any insight would be really appreciated.

A1: Assuming you've got valid reasons for viable air sampling, whatever your industry, there's nothing wrong with RCS's except a 'single-source' for the media. Many other samplers-- SAS, SMA, STA, etc.- use common RODAC plates or 15 x 100mm or 15 x 150mm agar plates, which are available from a hundred sources. They each have their pluses and minuses- I'd see if mfrs. might be willing to provide demo's or maybe even 'loaners' for you to evaluate in your facility- then make an educated choice based on what you see in your own hands.

The really important thing is, there ARE differences between them in efficiencies, etc-- they are NOT truly standardized-- so, "CFU/Cu. Ft." with one DOES NOT necessarily equal "CFU/Cu.Ft." with another- so be prepared, if you change, to re-establish your baselines and Alert limits based on the sampler you end up using!

A2: A few years back I wrote a book chapter on this subject that may be of some help.

Vellutato, Arthur, Jr., "Air Samplers", Davis-Horwitz Publishing, Laboratory Validation, Editor Jeanne Moldenhauer, Chapter 18, P. 219-268.

A3: Why do you want to change RCS Air Sampler without any reason. I have been used this sampler for 15 years and no problem found on operating and test result. If you want to change you can introduce SAS Air Sampler in your Lab. It is cost effective, for this equipment, no require any special reagent or strip. Normal 90 mm nutrient Agar/SDA plate may be used for environmental monitoring in production area.

You can use the above equipments simultaneously in your Lab. for verification of you test result. I use the same manner.

A4: I wrote a review of the differences betweeen different type sof air-samplers:

Sandel, T. ‘Active Air Sampling – The Selection Process Made Easy’, Pharmaceutical Manufacturing and Packaging Sourcer, Winter 2005, pp30-33

A revised and expanded version will appear in the next edition of the European Journal of Parenteral and Pharmaceutical Sciences. Hopefully this will offer some guidance with the selection process.

Neutralization of hydrogen peroxide solution

I am trying to validate the efficacy of a sporicidal agent, 6% hydrogen peroxide solution, but I seem to have a problem in achieving the neutralization.

I have been using Lecithin 3g/l; polysorbate 80 30g/l; sodium thiosulphate 5g/l; L-histidine 1g/l; saponin 30g/l; in phosphate buffer 0,25mol/l at 1% as a neutralizer, but it does not work. I don't want to proceed with the membrane filtration, because this would totally change the method I have been using (spread method), and to be honest I don't think that I would make any difference.

A1: Please try the inclusion of Catalase in your neutralizing solution.

A2: D/E neutralizing broth is an effective neutralizer for Hydrogen peroxide.

A3: Try catalase?

A4: Try adding catalase (you can purchase a powder made from A. niger). We used to neutralize 3% peroxide with it. You will need to determine how many units of catalase to add per mL of media, but it does not take much - a few hundred units per mL is usually enough.

A5: Regarding the neutralization of hydrogen peroxide with catalase, I have been seeking for this enzyme and it seems that I can find it only in Sigma. Does anybody have any idea where can I get it from and if yes would it be possible to suggest a catalogue number?

A6: I purchase catalase from MP Biomedicals, LLC (phone 440-337-1200). The catalog number is 100402. This solution is prepared from Aspergillus brasiliensis and formulated to contain a concentration of the enzyme that is > or = to 1000 enzyme units per mL. The lots that we utilized were approximately 1100 enzyme units. The solution is not sterile and can be sterile filtered easily.

A7: If you have access to the large vendors such as VWR or Fisher Scientific, they should be able to help you. I believe that the manufacturer of the Aspergillus catalse powder we used to purchase was Calbiochem, but not sure. We definitely used to purchase it through VWR though.

A8: I have complete M.Sc. in Microbiology in June,2010.Currently I am working in Vaccine Production Department. But as I have done M.Sc. in Microbiology which is good for me either working in production or in Quality control department.

A9: EMD Chemicals offers a suitable catalase for this application:
http://www.merck-chemicals.com/usa/life-science-research/catalase-aspergillus-niger/EMD_BIO-219261/

A10: We get out catalase from Bio-Cat, INC located in Virginia.

Penicillinase activity

I also have a question about penicillinase solution. Following my method, it is necessary to add 2,5mL of a 107 KKU/mL to 5 mL of my product dilution. Researching, I found different values to convert "Levy Unit" to KK Unit. Can you help me? I have in the lab "Penase" in a 50000 UL/mL.

Reporting confluent growth

I'm wondering how other companies report environmental results that are confluent. TNTC isn't a great way to report when a subsequent study shows that the confluency can be the result of a small number of organisms.

A1: If you mean by confluent that the organism spread out over the plate, you count the spreader as 1 CFU and notate spreader on the paperwork. The FDA web site microbiology manual says to do this. Most organisms that are spreaders come from floors.
Paenibacillus is one. It does not seem fair that an organism can take up the whole plate and be counted as 1 CFU, but the manual says to do this

http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/UCM063346

3.Spreaders. Spreading colonies are usually of 3 distinct types: 1) a chain of colonies, not too distinctly separated, that appears to be caused by disintegration of a bacterial clump; 2) one that develops in film of water between agar and bottom of dish; and 3) one that forms in film of water at edge or on surface of agar. If plates prepared from sample have excessive spreader growth so that (a) area covered by spreaders, including total area of repressed growth, exceeds 50% of plate area, or (b) area of repressed growth exceeds 25% of plate area, report plates as spreaders. When it is necessary to count plates containing spreaders not eliminated by (a) or (b) above, count each of the 3 distinct spreader types as one source. For the first type, if only one chain exists, count it as a single colony. If one or more chains appear to originate from separate sources, count each source as one colony. Do not count each individual growth in such chains as a separate colony. Types 2 and 3 usually result in distinct colonies and are counted as such. Combine the spreader count and the colony count to compute the APC.

A2: Confluent Growth vs TNTC Good question. In case of bacteria, confluent growth by small number of organisms should be easily recognizable in their early stages of colony development. Example some Bacillus sps [cereus which has spreading colonies, some may be mucoidy, but they do not start off as mucoidal]. It is always advisable to look at the plates within 24 to 48 hours and not wait 72 hours because a method calls for recording the results after 72 hours. Once you know a colony has appeared within 24 hours, make a note and keep a watch and see if it is spreading. Then you count that as single colony after 72 hours even though it is spreading!

In case of filamentous fungi, like Aspergillus niger, again one has to understand how they grow and sporulate. Do not wait to count fungal colonies after 5or 7 days [although most methods calls for recording the results after 5 or7 days]. Catch them on plates before they start sporulating. Because once the colony starts sporulating, the spores tend to dissipate within the plate and will give you an artificially increased colony count when in fact your product or original sample may have just one or two spores! I note that many people make this mistake in reporting fungal contaminations in products. Again this is a matter of checking the plates before it is too late!

If one understands the colony morphology and characteristics of microorganism in general, It should not be difficult to differentiate a TNTC vs confluent growth.

Water system Validation

I have a question regarding Water system Validation. Before I ask the question- here is the background. We manufacture topical, non sterile medical device/drug products. We manufacture on an average three times a month (could be twice in a week or just once a week). We have been using water system for over 8 years which has yielded USP Purified water to meet the specification. We send out a sample for TOC testing every time we manufacture and test for micro and conductivity in house. The samples are taken just prior to adding water to the batch tank, and immediately after the water addition is completed to assure that water that went into the tank is tested for USP purified water criteria. We have also tested all sample ports on a monthly basis to ascertain that each filter or DI bed or UV light performs as intended to. We only have one point of use.
As mentioned, the system has consistently produced great results when maintained according to procedures (sanitizing, UV light change, filter change and is recirculated 24/7)
Now we are preparing validation protocol. My question - based on all the data we have- I was thinking of testing as follows:
daily for 2 weeks
twice a week for 4 weeks
once a week for 12 weeks
and then every time we manufacture for the rest of the year to address seasonal variability (which is already done as we have data for all these years)
Hopefully we would be manufacturing more than the above mentioned schedule and test when we manufacture. Can I use that data for validation, without taking an additional sample that day or week?

A1: Regarding your query, you have not mentioned whether there is a storage tank for purified water inbuilt into the circulating system. I would suggest the following validation program :

1. All sampling points and user points - daily for 4 weeks (chemical and microbiological testing). The extent of chemical testing from each point should be appropriate to the purification stage being monitored. The storage tank should be included in this testing program.
2. Subsequent weekly sampling of all points, along with daily sampling of the user point. Daily monitoring of water from the tank should be carried out.
3. You should continue to identify and test the water specifically used for manufacturing of each product.
4. Follow the above schedule for 1 year after the initial 4 week testing period. This will give you your comprehensive seasonal validation data.
5. Carry out periodic trends reviews (monthly or at least quarterly) of the observed values of the critical control parameters - total viable count, conductivity (offline and online), TOC and other chemical parameters if tested.
6. Along with your historical data, the above data should give you sufficient information to fix effective monitoring frequencies and limits for routine monitoring.

Using blood agar plates for environmental controls

Can blood agar plates be used during environmental controls or are they selective for some type of bacteria/fungi?

My hope is that all microorganisms (both bacteria and fungi/yeast) that grow on standard TSA plates can grow on blood agar plates.

A1: Blood agar is to rich for EM. True EM organisms are slightly damaged and by using blood agar you may get false zero counts. If you only want one media, then TSA would be your choice.

A2: You can use whatever you can validate. However, be prepared to be challenged by auditors.

Blood agar is typically used for organisms living in/on animal/human bodies. Environmental microorganisms can include human bugs but also would include those from soil, water, plants, and other non-human sources. The blood agar is probably not ideal for growing these organisms, you need something with less salts and nutrients as your "everyday" EM media.

A3: I had experience with 5 % Sheep Blood Agar plates for environmental monitoring but not in Pharmaceutical clean room facility. It is normally used environmental monitoring for hospitals particularly in Surgical Theaters. Yes, The blood agar supports the growth of bacteria and fungi. The main advantage is we can detect pathogens like *Staphylococcus aureus*, * E.coli* with their beta hemolytic properties and colony morphology on Blood agar.,But drawback is very difficult to get sheep blood for preparing the media when compare toTSA.

So my point of view, TSA is enough for Environmental monitoring and no need Blood agar for EM.

A4: The primary reason that blood is added to TSA, is to determine hemolytic capabilities of organisms, which is used as an aid in identification. For the most part, it does not affect recovery rates.

A5: I would not recommend the use of blood agar as environmental controls over TSA becuase they aren't selective for yeasts and molds. You may face difficulty in recovering or sub-culturing isolates from water systems as well due to the nutrient rich media. SDA is commonly used for recoveries of yeasts/molds but many have shown TSA to be effective when being used in environmental monitoring or as EM controls such as settling plates or used in air ideal samplers.

A6: Based on your replies, I will not move forward with the blood agar plates and rather source TSA plates. That will ensure that we don't have an issue of false negatives and will save us a lot of extra validation work.

Medical Devices

Please can you help me with this 'auditor created' problem.

We currently produce an antiseptic first aid cream which is listed as a class 2 medical device. Having just been visited by an auditor I am now informed that as a medical device there are certain criteria which must be met.

I am told that a full validation of recovery percentages needs to be undertaken for the product (a cream), that having obtained these figures I must then go on to alter all my results by this 'conversion factor'.

My question is, I know recovery percentages and conversion factors are perhaps relevant to the more recognisable medical devices like sutures and joint replacement units, those types of equipment where surface recovery percentages (which is how I would recognise this)are obtained, but is applying recovery percentages and conversion factors for creams (which would be tested using standard broth based methods) something that any of you perform? Or even heard of?

A1: The auditor may be making reference to bioburden validation which is the validation of the product bioburden process as per ISO 11737-1. This is a required activity when performing validation of a sterilization process or when product bioburden is performed. So, this validation is performed when the product is enumerated for microbes. If you are not enumerating microbes in your product, you should not be required to perform the bioburden validation.

To explain a little further, bioburden validation takes into account that a bioburden test will always recover 100% of the microbes in and on a device, therefore a bioburden validation is performed to determine the percent recovery of the bioburden method. The bioburden validation generates a percentage of recovery that is actually achieved when the bioburden testing is performed. This percentage is used to "correct" the bioburden amount as the auditor mentioned to achieve the final bioburden.

I cannot determine from your email if you have a sterile product, or if this is for bioburden testing.

A2: If your test method is using a broth-based method (meaning a test where the product is placed into broth and evaluated for growth or no growth) then you are not removing the microorganisms from the product prior to testing. In this situation there is no need for a recovery efficiency.

The relevant standard for medical device testing for bioburden is ISO 11737-1:2006. In that document, determination of recovery efficiency is described as being critical, but that is only the case when removal of the microorganisms from the product is part of the test method. It appears that in this case the product itself is being tested rather than trying to extract the microorganisms from the product.

If I am misunderstanding the test method please clarify.

A3: You cannot legitimately perform recovery efficiency with a cream product.

If you are testing the cream by conventional means, such as TAVC it should include method suitability tests which will confirm adequate recovery of organisms after neutralization of any preservatives in the product. In my view this should serve as adequate data to demonstrate that any organisms that may be present will be cultured and found and therefore negate the need for any recovery efficiency work-up with a view to applying a correction factor.

As [name redacted] says, the ISO 11737-1 method is designed for those bioburden determinations where the organisms have to recovered from a hard surface and in this situation a recovery factor is needed.

I sympathize with your auditor created problem having had a similar issue recently!

API TAMC and TYMC

According to "5/.1.4 E.P. 6.7 and <1111> USP 32 - // Microbiological quality os non-sterile pharmaceutical preparations and substances for pharmaceutical use" /TAMC and TYMC must be determined for substances for pharmaceutical use. For APIs, has anyone performed a risk analysis strategy or evaluation/justification in order to avoid microbiological testing of every single batch?

A1: Without relating to the regulatory aspects of this point, and changing the shift of the topic somewhat, I would mention one incident that occurred in a previous workplace of mine, which manufactures non-sterile drugs.

The company did not have a policy of checking every incoming batch of raw material, whether API or excipients. Some years ago we produced a batch of tablets which was found to be heavily contaminated with mould. The contamination was traced to an incoming batch of excipient, a salt produced by one of the leading and most respected manufacturers in the world. Since then, the company has had a policy of checking every batch of incoming raw material used in production.

Do you perform micro testing on every batch of final product? In non-sterile products, it is common to check only every 5th batch or so. If you haven't checked your raw materials, you run the risk of letting a contaminated batch of final product slip through your fingers.

It is true that there are some APIs in which no self-respecting microorganism will grow, but some microbes aren't self-respecting. Ultimately, you have to decide whether the considerable effort involved in checking every batch of raw material is worth it. In order to avoid product recall, it may be.

A2: We are not performing the testing of each batch or lot in non sterile products we are checking 5th lot, 10th lot& 50th lot because it is not possible to check every lot / batch in non sterile product production

As per historical data of 100 batches /lots we are going on reduced procedure i.e. 5th lot, 10th lot &50th lot after 100batches WE are doing the statistical Analysis of these lots

If any problem or out of trend value comes then we test 4-5 consecutive batches /lot related to that product

If you want that no product recall comes in your plant then you take preliminary actions
1. If any change in trend of results then check.

A3: Suggest it is not common to test "only every 5th batch" of non-sterile finished product. In my experience, every batch is addressed.

Raw material testing is important but certainly is not a guarantee against finished product contamination.

using EM isolates for USP <61> suitability of the counting method

Will you send out this general inquiry regarding the use of EM isolates for prep tests. We are now beineg asked to include them for a particular client (recommended by the FDA).

A1: USP <61> details the requirements for growth-promotion testing of media and suitability of the microbial enumeration methods. The test microorganisms are listed in Table 1 of the genral test method.

If you include the compendial test in your regulatory filing you meet the requirements of the test by following the requirements outlined in USP <61>.

There are no requirements to use environmental isolates for either growth promotion or suitability testing. The recommended microorganisms are representative of other microorganisms that can be isolated using the recommended media and incubation conditions.

Gemcitabine Neutralization

I am looking for a way to neutralize Gemcitabine HCl during sterility test. Per literature and results of early method development, Gemcitabine HCl has very high antibacterial activity against Gram-positive microorganisms.

Non-viable Particulate Monitoring

Question: when you perform non-viable particulate monitoring with a portable counter, do you consider the paper printout from the counter to be the raw data?

A1: Yes I was consider the paper printout from the computer to be the raw data, because it is your evidence for the qualification of performance of your system to assess whether HVAC, Nitrogen or compressed air filtration filter, so that your insurer that is within your specifications in accordance with the rules you're using as a reference for your criteria or qualification specifications.

A2: Yes, printouts are signed, dated, attached, and filed. Since tapes are thermal printouts we photocopy the printouts for posterity.

A3: Unless you have a way to download the data from the particle counter to some software system and this software is validated, then the printout is the data. I am not sure what other options you have.

A4: This depends on your EM data system. The printout is typically considered raw data when you use a paper-based documentation system. If you download the data directly from the counter into a validated database then the printout may not be the raw data.

A5: Yes it is the raw data and the needs to be safely kept for record.

A6: Absolutely. We photocopy the raw data printout due the break down of the thermal paper over time. We have a full paper less lab system but still keep the printouts because it has been our understanding this is raw data.

A7: I have done the same thing in every Pharma establishment I have worked in.. for the same reason... it is considered your raw data.

A8: The paper tape would be considered raw data and should be kept. A copy should be kept with the associated batch records so that it can be reviewed by the Quality Unit.

Sanitization frequency

I wanted to know something about sanitization of Grade D areas. We have big corridors classified as Grade D, they are the corridors to the Isolators in Grade C areas. Can some one tell me the sanitization schedule for the floors of Grade D areas. Like How many times they do in a week and if they rotate any sanitizer.

A1: Cleaning and sanitization is typically daily as these are high traffic areas. However your HVAC PQ will give you more information for setting the times for cleaning and sanitization.

A2: Sanitation frequency in any Class is depended on your validation date such Effectiveness of cleaning agent, interval of sanitation, bioburden load, Holing time of machine or room after sanitation. If you have standard HVAC system, you will set your sanitation as per requirement. At that case, Bioburden load should be determined before sanitation and after sanitation, you have to verify your sanitation system. This is simple analysis. Frequency sanitation and rotation of sanitizer both are important issue for controlling of Air classification. Air monitoring(Particle Count and Microbial count),, swab test of surface area are important parameter to set sanitation frequency.

There are many articles in various website for cleaning Validation that you can download.

A3: The floors in ISO-8 cleanroom operations should be cleaned and disinfected daily and the walls and equipment at least weekly depending on the activities performed within each room. So, a disinfectant should be used on a daily basis or more frequently as room activities dictate and a sporicide should be used as needed to address fungal and bacterial spores. The usage of the sporicide should be based on the frequency and number of bacterial and fungal spores found as part of your facility environmental monitoring program.

sampling plan for bioburden

Anyone knows if there is a requirement to sample Beginning, middle and end of the filling to test for bioburden of a bulk product?

Or is there anything in regulation that define how many units to sample for a bioburden test of a bulk?

Also, anyone knows the minimum volume require for the bioburden?

A1: Yes, it required to sample from all 3 stage and minimum 100 ml quantity required for bioburden test.

A2: Where can i find those requirements? Do you know any reference?

Antibiotic assay estimate vs real world potency

Just for interest sake I was wondering how the antibiotic assay estimate produced in a lab relates to real world potency given the assay conditions are unlikely to reproduce those seen in or on a human at the point of use.

Fungal Contamination - Articles

I am searching published articles in the topics related to *'A profile of Mold contaminants in Pharmaceutical Clean Room facilities'* But only limited publications available in this topic. If anybody having publications in this topic, Please send the details, This will be very greteful to me.

B. cereus in non-sterile oral dosage forms

Can the group share their current perspectives on B. cereus in raw materials and subsequently in finished, non-sterile preparations for oral use? Does anyone specifically call out this organism as objectionable and if you do, for what types of dosage forms? Has anyone set up specs for absence of the organism and/or recovery levels? Knowing that the organism will not proliferate under an Aw value of 0.95 this should not be a concern in tablets and related preparations. B. cereus is in the FDA Bad Bug Book but if you read some of their guidance documents for infant formula and medical foods the concern is when the level of this bug reaches 1000 organisms per gram.

A1: I am glad you asked this question on Bacillus cereus in raw materials. I am also trying to understand why in the personal care industry there is a spec of <100 cfu/gram expected by many customers in raw materials that will anyway end up getting formulated in a finished product that would be preserved with a preservative. Even the guidance from FDA BBB for infant formula /medical foods [which are more critical] is set at >1000 cfu/gram .

Is the expectation of <100 cfu in the raw materials an overkill ! By the way, the raw material I am referring to is a plant based seeds that are harvested from the field and the whole manufacturing process is unsterile operation.

A2: It is a generally accepted principle that preservatives are not an alternative to appropriate GMPs. Thus the bioburden needs to be controlled in the raw materials.

The limits will depend on the intended use - they should be tighter for use in the manufacture of sterile products (terminally sterilised or aseptic manufacture/sterile filtration). For sterile products raw materials may be required to have bioburden levels of 100 cfu/g or less.

For non-sterile final products there could also be a need for control on the presence of certain pathogenic microorganisms. Obviously what can be pathogenic will depend on the route of administration and the general state of health of the patient. Hazardous pathogens should be absent from materials used to manufacture parenterals.

There is often guidance on the limits that are appropriate for non-sterile products in the pharmacopoeias.

A3: It is not "overkill". Finished product preservation is clearly not intended to address or mitigate raw material microbial content. For most cosmetics and personal care products, the finished product spec limit is 100 cfu/g so reapplication of that standard to raw materials is very reasonable, esp. as speciation of Bacillus spp. is not usually performed.

A4: I absolutely agree with you on the need for an appropriate GMPs for any products manufactured [finished or raw materials] and the need for checking on bioburden in a given material. Preservatives and biocides are not the solution for bad manufacturing practices. There is no substitute for good hygiene practices in plants and expecting reasonable quality raw materials.

I also agree to the specified/objectionable microorganism checks based on the finished products and the route of administration. Everything needs to be assessed in context. And you correctly state that a limit of <100 cfu/gram for medically significant sterile products raw materials is justifiable. It depends on the intended use.

Can you site a specific guidance documents specifically addressing B. cereus in cosmetics and personal care products that requires it to be below 100cfu in a raw material. I am struggling to find a reference on this. I understand the need for absence of specified organisms like Staph, Salmonella, Yeast, Mold, G-ve etc etc. in certain products depending on intended application.

A5: I have not come across anyone with a specific limit for B. cereus, though I have not done anything with infant formula or infant specific formulations in the past 10 years.

For [name redacted] comment, I will add that many spices and some plant seeds bound for food products are subjected to bioburden reduction processes before going to market. Some raw spices brought into the United States are processed with Ethylene Oxide using cycles designed to kill mold spores. I recall reading a paper about experiments using E-Beam on spices as well. (I just knew that odd bit of information would be useful some day!) It may well be that your raw seeds are processed in a similar manner. You should expect that the subsequent processing is "clean" and a low bioburden would be reasonable.

A6: B. cereus causes food poisoning at concentrations > 1 million/gram or mL. It does so by means of 2 different toxins. Finding B. cereus al low concentrations in raw materials should not be a cause for concern, unless you put its absence as part of the specifications.

A7: Can you clarify if we do the speciation and confirm that the isolates frmo raw materials are invariably100% Bacillus cereus that occurs ~1000 to 5000 cfu per gram, can that be acceptable since per another contributor in this string of responses [Jose Martinez] mentions that B.cereus is an issue when the numbers are greater than 1 million/gram. Just a thought!

A8: I agree with [name redacted] recommendation.

By the way he wrote an excellent article on bioburden
Martinez, J.E. 2002 Microbial Bioburden on Oral Solid Dosage Forms Pharm Technol. Feb 58-70

A9: Not sure if I responded so allow me to do so here. I'm not aware that such speciation is commonly conducted. As for the numbers - I'd look to the relevant specification.

Aseptic technique and Sterility Testing

I wanted to ask what kind of laminar would you suggest for sterility testing of samples.

A1: Your best sterility testing environment would be an isolator. There are any number of isolators designed specifically for sterility testing. If your product is too bulky or the sterile barrier is permeable to the sterilant gas/vapor used to disinfect the isolator, then an isolator may not appropriate.

I have always been partial to horizontal laminar flow hoods. I think it is easier to keep your hands out of the "first air" during testing in a horizontal flow hood than it is in a vertical flow hood, minimizing the potential for false positives. However, some of this preference changes depending on the type of product you are testing. If the product sample is any kind of hazardous material, or something that could aerosolize and "blow" into the face of the tester, then I would be working in a containment hood.

Defined Flow Rate for WFI systems

I have two questions about flow rate requirements for WFI systems. The first is if there is a specification that a WFI loop must maintain a specific flow rate of "X" fps or "X" m/sec?

The second question I have is if there are requirements are for recording and presenting the flow rate data from the WFI loop? I see in USP <1231> in the "Monitoring Program" paragraph that "The program may include a combination of in-line sensors or automated instruments (e.g., for TOC, conductivity, hardness, and chlorine), automated or manual documentation of operational parameters (such as flow rates...". Does this mean that the owner of the WFI system must be able to demonstrate what the actual flow rate of the WFI system is at any given point in time, or just have documentation that at one time (e.g. during OQ) that the flow rate was established and met as a requirement of validation? I'm trying to determine if the requirement for documentation means that one only has to know that the system has a flow rate via in line monitoring or other means, or if continuous monitoring must be performed to show the exact flow rate as a number value.

P. fluroscence

I `m looking for a method to detect Pseudomonas .fluroscence.(can we use USP 62)your input would be appreciated.

REGARDING THE VALIDATION OF WATER BATH

Can i validate my water bath using Biological indicators. if i can plz let me know the biological indicators used for this validation.

A1: I was wondering what temperature your water bath would be set to? And what it is going to be used for? I am not too sure about using bio indicators as a validation method for a water bath. My main concern would be the consistency of the temperature over a period of time, water quality being used in the water bath and also there is a huge concern of micro organisms that like to contaminate water baths (depending on the temperature) so the use of a water bath detergent would be a good idea, plus regular cleaning :)

A2: The instrument of water path doesn't consider to be sterilized or to determine the number of log reduction in contaminants. cause the temperature and exposure time are not previously determined.so you don't need to use biological indicator in this instrument cause any of figures would be variable there are no constant factors. I recommend you to use specified disinfectant with the water you will use in this operation that will minimize the extrinsic growth.

A3: I am having a manual controller hot plate in my lab which is having three heaters. Is is necessary to show the calibration?If yes, can anyone suggest how to calibrate it??

A4: No regulatory needs to validate your water bath but the sensor should be calibrated. If there is an digital display with attached sensor then it should be calibrated and if not then you can put calibrated thermometer and check manually. The most important thing is that you should keep a log of temperature recording. If your sensor is working fine then why you need validation. Rest you should also clear your concept of validation and calibration that what should be validated and what should be calibrated, it will solve your query.

A5: Take a look at USP chapter "<1058> Analytical Instrument Qualification" for some guidance on this. This new USP informational chapter is a good reference for micro labs in their need to gain some control over the expectation of equipment qualification and how to accomplish this.

One of the interesting aspects of this chapter is the description of the qualification requirements for different types of equipment based on their relative complexity and uses. I believe, using this approach, you could easily justify restricting your concern to a study of the controllers and the ability of the bath to maintain a set temperature (if you even want to go that far).

As an aside, there is additional information in a new reference book "Laboratory Design: Establishing the Facility and Management Structure" - information at http://bit.ly/b5r2ZY.

Bioburden for radiopharmaceuticals/PET agents during clinical trials

FDA has said that they do not require a bioburden study (since in process controls are not possible) for aseptic PET products during clinical trials but they will require it for an NDA.

Does anyone have any experience about what other regulators/pharmacopoeias etc. require?

PET is kind of different due to short shelf life and the guidelines do not say anything about this creating a difficult position for many drug developers, especially in early phase.

Can a risk assessment approach be used where critical steps in the PET process are evaluated?

Does anyone have experience with this or similar short lived radiopharmaceuticals?

I feel that demonstrating low bioburden prior to the sterilizing filter is very important since in process controls are not possible.

M7H9 agar plates for AOAC Tuberculocidal test

I don't know if anyone here has experience with AOAC 965.12 Confirmative in vitro Test for Determining Tuberculocidal Activity. I need to make Middlebrook M7H9 agar (M7H9). This agar requires the addition of Middlebrook ADC Enrichment aseptically post sterilization. Once these plates are then poured, does anyone know the recommended shelf life when stored at 2-5C.?

A1: In a previous position, my lab had validated solidified bottles of Middlebrook 7H9 agar (360 mL each) without enrichment to have a shelf life of 3 months at RT. The Middlebrook ADC (for agar) & OADC (for broth) enrichments were validated to have a shelf life of 1-month under refrigerated storage conditions. The solidified agar would be melted in the autoclave and 10% enrichment would be added once cooled to 45C.

We typically would use the poured plates on the same day. All long term storage was confirmed in bottles prior to addition of enrichments.

You would obviously want to validate the shelf-life for your own lab.

A2: I can't give the answer to your question since I have never worked with this media. However, you can set your own expiration date. Start by making the media and then perform growth promotion test at time 0, 1 week, 2 weeks, etc. As long as your media meets growth promotion test requirements - you can extend the expiration date.

A3: We also have a 30 day shelf life on these plates but normally use them in a much shorter period of time.

A4: We usually order this media either through VWR or BD. That works out much better if you accept the vendor's C of A for the same.

You could also call these suppliers and talk to their Technical support team regarding your queries. They will be able to provide you with the required documentation as well.

WFI by ultrafiltration

I want to know if anyone have any experience producing WFI using a ultra filtration technique.

We have establish a PW system which have a pretreatment, reverse osmosis and CEDI.

Inspection Process for Laboratory materials

I am trying to gather some information regarding the need for the process for the inspection of laboratory materials. In most ISO companies there is the need to develop specification documentation regarding each item ordered and approval of the would-be supplier(s).

I am of the opinion that once you have your approval for these suppliers that there is absolutely no need to approve the individual manufacturers who supply your approved supplier. Does anyone agree with this?

In addition is there a need to have inspection levels on laboratory materials (petri dishes, membrane filters, pH buffers etc) and specification documentation on EACH shipment of these items from an approved supplier? I am unclear on this issue and any help/suggestions/comments would be much appreciated.

Storage of running ATCC culture

Is it possible to store ATCC cultures on solid agar plates for > 1 week at 2-8C for the use in growth promotion testing? This is in the event there is not the option of using -80C freezer for cryopreservation. If yes is there a defined time period when these strains can be held at this temperature (2-8C) before they become unacceptable?

A1: The concern here is not the viability of the organism. In an agar plate, that is not air tight, or even sealed using non-sterile material like (parafilm) one can not guarantee the purity of the culture. If you have a procedure in place that will allow you to check the purity of culture, that should be fine. Alternatively you can have the organisms in a low nutrient liquid media in tightly sealed tubes.

A2: It is possible, when you order or receive anything from the ATCC you receive with that a guarantee that these cultures are not only what they say they are but that they are pure and are the strain indicated. Along with that they should give an expiration date.

http://www.atcc.org/CulturesandProducts/ATCCGenuineCultures/tabid/616/De fault.aspx

Now, when it comes to keeping these cultures for more than one week there is a little bit of work involved. For the most part all organisms can be kept for at least 4 single and separate generations. If you take your ATCC culture, plate it and incubate appropriately, you will then have a "mother culture" this is the most pure form of live organisms in your possession. The mother culture is never used directly with any test to avoid contamination and it is stored appropriately for 4 weeks. From this you can take a subculture once a week by streaking an inoculation from the mother culture and incubating appropriately. Once grown, you can harvest as many times as needed during this week (which we label as passage 1). When storing, do not store in the same area as your mother culture as they may contaminate the pure cultures. You can repeat this 4 times with the same mother culture. You can successfully make your stock last a full month however, after this time you run the risk of losing viability. The reason for not sub culturing from your passages/first subculture (single generation) is because you risk losing integrity by mutation of the organisms after the first generation from the mother culture.

There is plenty of info online about sub culturing and mutation after the first generation.

A3: The agar plates would be sealed with Parafilm to minimize contamination. Is there a maximum time for keeping ATCC cultures at this temperature on agar or low nutrient liquid media? Which low nutrient media types would you recommend?

A4: Not all ATCC isolates come with an expiration date. Majority of the freeze dried pellets will not have an assigned expiration date. In any case all shipments from ATCC should come with a "Product Information sheet" something like technical information sheet which states the ATCC #, Organism name, Citation of the Strain, Recommended ATCC -Reconstitution or Propagation media and all that good stuff.

A5: The mother cultures are usually stored refrigerated but in a separate part from the running cultures. The storage of the mother culture does not extend beyond 4 weeks? What if the cultures remain viable based on growth promotion testing and also culture and microscopic investigation?

The USP does state that the cultures should not exceed 5 generations from the stock culture. I do understand the risks involved in subculturing leading to mutations and strain variations. However if the first mother culture is subcultured to make another mother culture then that second mother culture is 2 generations from the ATCC strain. Any subsequent subcultures from this second mother strain would be generation 3 and should still be within the USP and ATTC guidelines.

A6: As per ATCC technical bulletin, ATCC cultures should not be stored more than a week on agar slant keeping at 2-8 C.
The reason behind the recommendation is, some cultures may not remain viable especially fast growing culture with less generation time.

If you follow one week time, SCDA/TSA or NA can be used.

A7: If its to be stored for more than a month or so then its preferred to store at -80deg cel.

Coliforms, Enterobacteriaceae, and E. coli

Today I was posed with a question from one of our vendors, and it is in regards to Coliforms and E. coli testing.

The vendor does not test specifically for E. coli on the grounds that they test for Coliforms and Enterobacteriaceae. They state that since E. coli is part of the group of Coliforms, then they see no need to test for E. coli. We, on the other hand, test for E. coli, Coliforms, Enterobacteriaceae, and E. coli o157:h7.

Do they have a valid argument?

A1: What are the respective methods?

A2: Technically, the vendor does have a justification stating that E. coli is a coliform bacteria and a member of the Enterobacteriaceae. However, you have to remember that if enrichment is being perform to isolate this particular bacterial species is what I call a "presumptive isolation" of E. coli. I would recommend to the vendor that final confirmatory tests will need to be performed on recovered isolates that are obtained from Enterobacteria Enrichment Broth Mossel, Violet Red Bile Agar and MacConkey Broth if you are conducting a USP Microbial Limits Tests in order to confirm what is the actual species that is causing a positive growth result in these broths and agar. For example, I have found Gram-positive bacilli species to cause gas production in Lauryl Tryptose Broth tubes used in a standard total Coliform Fermentation test for conducting the analysis of water and food samples.

A3: I forgot to mention that we use Current USP Methods <2021> for Enterobacteriaceae and <2022> for E. coli. For Coliforms we use AOAC 2000.15. The vendors use the same methods we use. Let me add that the products we receive from vendors are raw materials (mostly powders) to be used in the making of our products.

A4: Technically speaking, yes they do. Escherichia and Enterobacteriaceae are both coliforms (found mainly in the colin) as well as Citrobacter, Hafnia, Klebsiella, Serratia.

As it was said to me... "E. coli is a coliform, but not all coliforms are E.coli"

So when you say that you test for "E. coli, Coliforms, Enterobacteriaceae, and E. coli O157:H7" you are redundant. We test for specific coliforms because some are more dangerous than others like O157:H7 which is a specific strain of E. coli. If you wanted them to test for specific coliforms, ask for confirmation testing of your positives for E. coli & Enterobacteriaceae. Just because they have a positive for coliform growth does not confirm E. coli or Enterobacteriaceae are present! As an example, when doing environmental testing, if coliforms are found we would do a lactose broth test, if the broth was positive we would confirm E. coli with EMB (2 extra days).

If you are using this outside company, make sure you request confirmation testing on you coliforms and be specific on what you want. If not they will continue to give you the same answer. If they do not do confirmation testing to meet your specific needs... find someone else! As a vendor they have an obligation to show you their procedures, if nothing else schedule a vendor audit/tour to find these things out!!!

Pathogen presence/absence

How we can justify absence of a pathogen after retesting , while a presence was observed during the initial testing?

A1: Unless you can show the procedure used is invalid, or can explain it as the inherent error of the test for which you have verification procedures or a problem due to sampling issues, you cannot justify it.

A2: It is my assumption that a larger sample size was tested for the retest than what had been tested initially. If not, you are going to have a very difficult time in justifying the absence of the contaminating organism in the retest result. Furthermore, an Out-of-Specification investigation should had been conducted to determine the root cause of the initial reject. If the objectionable organism was present in the initial test and not in the larger sample size for the retest, It might be due to many factors. If the sample material was a raw material, it common for a contaminating organism not to be distributed homogeneously when the initial and retest samples had been taken for analysis. This homogeneously distribution can also occur with finished product.   If the material was a finished product or raw material , you have to rule out that the initial result was due to a laboratory error. This should be part of your Out-of-Specification test investigation by looking at the negative test controls and air sampling plates when the product was tested. Hopefully, the positive controls of the retest were able to detect the presence of microbial growth. You definitely want to rule out the possibility of a laboratory error in the retest results that prevented the detection of the organism. Finally, I do agree that preservatives should not be used to clean up microbiological issues in the manufacturing plant. However, it is very possible that the preservatives in a finished product finally killed the objectionable microorganisms before the retest was even conducted. It is my understanding that PCR is able to detect the presence of DNA from dead organisms in a finished product matrix and could be used to confirm this. but most QC laboratories are no equipped to perform this type of testing to demonstrate the viable organisms had been present in a test sample. However, this type of occurrence in which the preservatives in a formulation act before a retest is conducted is what I call "A Fact of Life" when retesting of items are conducted. Based upon the test data from your out-of-specification investigation, you have to come up with a reasonable justification as to why a sample was released based upon the retest results of a sample in which the initial test result was not in compliance with your microbial specifications. Finally, I do not recommend that a justification state that the preservatives killed the contaminating organisms and this was the reason as to why it was released.

A3: Only when there are preservants in this product you can justify the absence in retesting. If not, you should not even repeat the test, unless you are sure the contamination is not in the product but introduced during sampling, analysis, use of contaminated media.

A4: It sounds like you got ahead of yourself. What I mean is that you can only retest if you can justify that the initial test was invalid. You don't automatically retest and then decide which result to use. It sounds like you need to understand why the test was repeated.

A5: You do not mention if the product is a liquid, sterile, or non-sterile API or finished form?

For powders and solid oral dosage forms I do not believe a retest can be used to justify the the initial result of detection. I say this because contamination is not uniform in these kinds of products. One can find contaminated material in one container of an API powder and not in another. If it is a liquid product then I would suspect contamination to be more uniformly distributed. Obviously for a sterile products any detection is unacceptable.

A6: No I did not get ahead of myself.our client has asked for retesting on their product which was contaminated with E.coli (natural powder), restesting was negative .I can argue the possible non-uniformity of the product since the investigation report shows the product is contaminated.My request was if we can justify the result in another way.

A7: As a testing lab - you can only offer that an investigation found all operations, controls etc. as appropriate (or words to that effect), assuming they were found to be such. This should have been the response to the initial finding. Retesting was not appropriate - tho' I understand you can't reject instructions from a client.

Offering non-uniformity in this context, however intuitively obvious, needs data.

Stick to the facts.

A8: There are 2 things: Retest and Resample
If you get unsatisfied result you have to retest the same sample for rechecking your result. If you get the same unsatisfied result you have to do investigation including resampling, lab errror, analyst effect............etc.

A9: What is "unsatisfied"?.
You have to investigate if the cause is a lab error before thinking in a retest.
In general objectionable microorganism are in a very low concentration, and in general, if by chance you could detect it in the original sample, it is very difficult to detect it again in the retest.

A10: I misunderstood your question. Now I think I better understand your situation. It sounds like your client is questioning the validity of the results where initially they were positive and on retest were negative. I think your rationale that the product is non-uniform is the best you can make.

Container closure integrity test

We have a method to perform container closure integrity test immersing media fill vials during 15 minutes in a 10e5 CFU/ml Brevundimonas diminuta suspension and puncturing one of the stoppers with a 250 μm diameter needle as positive control. After that we incubate the vials for 14 days at 30-35C. This method used to work well for us until we came across one product that doesn't show growth in the positive control even after 24 hour of immersion.
Can you help us improve the method or suggest a different one?

A1: Could the product be somehow inhibitory of B. diminuita? Did you do neutralizer efficacy tests? Perhaps you need to inactivate some component of the product so that B. diminuita can grow. Even if unpreserved, sometimes some chemicals can cause stasis in certain organisms.

A2: We are also used to test CCI of our vials through Microbial Ingress Testing. I am not surprised there is no growth with a punctured stopper as this last is supposed to withstand the introduction and the removal of a needle. We worked a few years ago on the validation of a MIT method, where the sensitivity is demonstrated with a calibrated microcapillary, which works well so far. I don't know how to share this method easily on this forum but for information this method is publicated in the following journal: "Rational for the validation of container closure systems", O. Chancel, L. Pisarik, STP PHARMA PRATIQUES -

volume 17 - N° 1 - janvier-février 2007. Please feel very free to get in touch with me (please find appended my electronic business card) should you need any further information.

A3: Out of curiosity (I only have experience performing CCI testing with dye):

Why must you prepare your positive control as part of the "test"? By that I mean, is your positive control's purpose to show "if there is a 250 um hole it will be contaminated in this situation", or is it to show "this is what a contaminated vial looks like, so therefore any vial that looks different than this is negative"? If it is the latter, could you not just spike the vial with the bacteria directly and just examine it next to your test vials?

Why B. diminuta? Just because it is small, or because it is this small, motile, Gram-negative organism the greatest threat to your product?

Is there some sort of mixing or pressure/vacuum that your tested vials are subjected to? I am imagining a water-bath sized container full of turbid TSB that you have floating vials in, and one of those vials has a little needle sticking out of the septum to allow it to (hopefully) mix with the turbid environment. Could the needle not be submerged? Or perhaps the vial is upside-down so the turbid media can't displace the air (like a miniature diving-bell)?

Please forgive my ignorance of this test method for CCI testing - I am still relatively new to pharmaceutical microbiology!

A4: We have also noticed this problem when we were developing a container closure method. So we added a plating step to our procedure, where after the test is done, an aliquot of the controls and samples are plated out on an agar plate (or you can membrane filter the entire content). Positive controls that didn't show turbidity had counts in them when the content was plated on an agar plate. I've noticed that the size of the test vial and the volume of the product inside the vial changes the ability of B. diminuta to make the vials completely turbid or not. But lack of visual turbidity doesn't mean it's not there.

A5: You may need to use a smaller diameter opening for a positive control - 250 micrometers is pretty large in relation to a microbial leak. You might consider using a smaller opening for your PC. Also, FDA may ask you for documentation of the sensitivity of the test - that is how small a leak can you detect.

A6: We use media fill vials used to validate the aseptic filling of the product in order to have vials prepared exactly in the same way as the product but filled with culture media.

A7: We prepare a positive control in order to see whether the culture media will show growth in case the container closure is not intact. I'm not absolutely sure, but in the first place we were suggested B. diminuta because of its small size. I can't tell if motility is an issue in this test.

There is no mixing nor pressure in the test. We prepare the suspension and immerse the vials upside-down in small containers with enough suspension as to cover the container closure. It is all the same suspension prepared at once, but the vials are in pairs or groups of three. The idea is to see if the microorganisms are able to enter the vial, not to allow the suspension to enter it.

A8: The CCIT microbial ingress testing may show absence of growth because of 1. Problem in Media remedy -check GPT 2. If the GPT is ok and this is the problem specific to product then chances are that your drug product is bactericidal in nature. Try bacterial viability study by using B. diminuta. 3. May be your testing methodology have some inhibitory effect on microorganism or no intrusion of the media.-remedy-try to inset capillary in positive control to have more passage of media.

A9: You may need to leave the 250μm diameter needle in place in the positive control for the immersion portion of the test. It could be that the material that you are puncturing is "healing/closing" once the needle is removed due to the nature of the material from which it is fabricated (for example, amber caps on injection sites are designed to maintain their integrity after needle puncture).

queries about new facility

For our new facility ,we are going to qualify the steroid, non-Steroid & large Volume parental area. Clean room & microbiological aspect we have to monitor the Environmental quality to qualify and to ready for sterile production. So, I've some query regarding this new sterile premises qualification/ validation.

The queries are:

1.Microbiological laboratory would be handover after the production area. So we will do microbiological operation/ analysis/environmental monitoring etc. at our other microbiological laboratory (distance 5Km and 30 mints by car):

Is this feasible method?

b) Do we need transport validation i.e temperature control of Van, microbial recovery after transportation?

2. What will be the required Sampling frequency and duration for environmental qualification of new premises as per PIC/s or EU GMP guideline?B'cos USP say several weeks.

cross contamination

In pharmaceutical industry. Is it better to detect for cross contamination by beta lactam antibiotics in other antibiotics and pharmaceutical preparations by HPLC or by microbiological techniques.

A1: I would have thought that it would be better to avoid the possibility of cross contamination by using a dedicated facility in the first place.

A2: In the pharmaceutical industry the manufacturing of beta lactam drug products should be segregated by building and preferably by location.

The milk industry used microbiological methods, e.g. penzyme and charm systems for screening down to a 5 ppb level and HPLC for confirmatory testing.

regarding validation of autoclave and DHS

Actually as per the guideline the validation of Autoclave and DHS will be done by running three successive cycles for each pattern of loads. however how come it is correct that running only one trail for each patern and completing the validation.

Shall i know the references for this query. is this necessary that each time when we validate the eqipment 3 trails must be performed or even one trail is enough?

A1: This is regarding Validation of DHS and Autoclave running 3 trials is better to ensure all the parameters should comply as per the given specification with reference to guidelines.one trial may also be sufficient because in media fill we are going dry phase 1 trial and wet phase 1 trial during periodic validation. during initial qualification 3 trials we are doing.

61 MICROBIOLOGICAL EXAMINATION OF NONSTERILE PRODUCTS: MICROBIAL ENUMERATION TESTS

Does <61> apply to growth promotion/enumeration of media used for environmental monitoring purposes only. (i.e. TSA 15x100, TSA 15x150 and TSA contact plates). More specifically,do we need to comply with the following subsection?

For solid media, growth obtained must not differ by a factor greater than 2 from the calculated value for a standardized inoculum. For a freshly prepared inoculum, growth of the microorganisms comparable to that previously obtained with a previously tested and approved batch of medium occurs. Liquid media are suitable if clearly visible growth of the microorganisms comparable to that previously obtained with a previously tested and approved batch of medium occurs.

We comply with growth promotion requirements for all media used to perform the Microbial limits challenge, including TSA when required. We are currently doing it for all incoming media regardless of intended use. I didn't know if we could separate the requirement for media used to test non-sterile products as opposed to media used perform EM and implement less stringent requirements for growth promotion of EM media.

CCI test

I have some comments to make on CCI test. It is important to ensure that the challenge strain(Brevundimonas) used is of very small in size; this is possible by using the selective media for the challenge strain. Then, It is ideal if there is a way to puncture one of the vials with a 0.3 micron needle if possible as a positive control. As this may not be possible, the firms have been using the needles of different sizes 250microns /300microns for creating positive controls. I am also not a big fan of incubating the vials for 14 days. Incubation for a period of 7 days at 30-35C is adequate for this application. After all, the bacterial challenge strain needs not more than 3 days for the growth.

Would also like the firms doing CCI test to comment on the below to benchmark the test method.

Do you use the selective media for Brevundimonas?

Do you ensure the size of the challenge strain before use? If yes, how? By microscopy or by filtration through 0.45micron ? How do you run the positive controls? What diameter of the needle is used?

Do you immerse the positive control with needle in hte solution? Or just puncture and remove the needle and immerse the positive vial in the solution? How do you justify the use of needle diameter of 250 micron? When do you perform the test along with the incubation of media fill vials? or after completion of media fill vials incubation? Is the test only done for media fill vials? Is it also done for product vials? Is the test done for every batch of the product? or whenever there is a change in the container /closure configuration?

Testing ether-containing material

An industry colleague of mine who is not on this list was asked about microbial limit testing of a non-sterile product containing about 60% ether and 20% unspecified alcohol. It was suggested from "higher up" that the test should be performed. Whereas we understand that 20% alcohol may act as a preservative, we found it difficult to see how anything would survive in 60% ether.

Can anyone shed any lihjt on this, preferably with references?

A1: As spores - bacterial and fungal (below ascospores) - you could have survival.

Selective killing of vegetative cells in sporulated yeast cultures by exposure to diethyl ether
IW Dawes, ID Hardie - Molecular and General Genetics MGG, 1974 - Springer
... At a concentration of 30% ethanol there was some differential survival of ascospores compared with vegetative organisms but to a ... Ether Selection of Yeas~ Ascospores ... Waites, WM, Kay, G.,

Dawes, IW, Wood, DA, Warren, SC, Mandelstam, J. : Sporula- tion in Bacillus subtilis. ...
Cited by 69 - Related articles - All 2 versions

TESTING DESINFECTANTS

I´m trying to make a validation protocol in order to validate my sanitization procedure. And I´m using the AOAC methodology as reference for these tests. And I'm suppose to use "carries" (polished stainless steel cylinders), 8 +/- 1mm od, 6 +/- 1mm id, length 10 +/- 1mm.

Does Somebody have experience in these kind of analisys? And known where I can find these carriers to buy?

A1: Try searching online for "Peni Cylinders".
Here is one source:
http://www.biologics-inc.com/inhibition_zones_peni_cylinders.html

A2: I am currently doing routine work with the AOAC Use Dilution hard surface disinfectant efficacy testing using these carriers. What testing are you doing? For bactericidal testing, use stainless steel penicylinders available from S&L Aerospace Metals, Maspeth, NY or Fisher Scientific Cat No. 7-907-5. For Tuberculocidal testing, you must use Porcelain penicylinders available from Presque Isle Cultures.

For more info: Official Methods of Analysis. 2009. 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, (Methods 955.15 and 964.02).

Official Methods of Analysis. 2006. 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, (Methods 955.14).

A3: The carriers are available from Fisher Scientific laboratory supply house. They may be listed as "penicylinders" as they are also used for MIC testing and antibiotic sensitivity tests. The test methodology is analyst sensitive so some practice with the method may be required.

A4: You can find these carries at Stamping& Washer Specialists (BOKER'S, INC) and the part number is 1875 XNANE X-060 and the quantity is 100/box. Their phone is 612-729-9365.

A5: You would want to use a compliment of facility surfaces that exist in your operations so you can discern the effectiveness of the agent against a barrage of facility isolates. Normality is 5-7 surfaces and 5-7 organisms. Using just stainless is problematic as effectiveness of disinfectant on porous surfaces such as vinyl, epoxy, kidex, mipolam and plastic is less than stainless. The pores harbor organisms and the disinfectant cannot get to them. Also the AOAC methods are for registration of a disinfecting agent in to a regulatory agency by a manufacturer of a disinfecting agent so that they can make antimicrobial claims for the product. It is not preferred for validation in pharma and biotech. Preferably would be a simple time contact kill study. It can be tailored to what is actually being done. The AOAC is tough to perform, expensive and riddled with inconsistency.

A6: Just a reminder that I forgot to mention before. You need to prequalify all your cylinders prior to use if you are doing "official" AOAC testing.

A7: The most common and accepted method for evaluating disinfectant efficacy testing is a carrier test on common substrates in your cleanroom such as lexan, stainless steel, kydex, epoxy, vinyl, and terrazzo. You can obtain coupons for testing from vendors such as:
http://www.alspi.com/coupons.htm
www.mcmaster.com

There are some current ASTM methods such as the QUDT that can be modified for efficacy testing.

water system revalidation

Is it require to revalidation of the water system which is in continuous state of the operation ,the water system had already finish with the phase 1,2 and 3 validation cycle and as a part of the validation master plan this revalidating was ask to plan, i need opinions of the forum members on this. if it require do we have to go for all the POUS and how long we have to go for the validation. and is their any reference for doing this available?

Reliable source of general microorganisms information

Does anyone know a reliable source (website, database, book...)to find general microorganisms information (morphology, nutritional requirements, habitat, pathogenesis).
I'm specially interested in microorganisms normally found in environmental controls of manufacturing facilities.

A1: I always used and preferred the gold standard: Bergeys Manual of Systematic Bacteriology.

A2: try this link
http://www.fda.gov/Food/FoodSafety/FoodborneIllness/FoodborneIllnessFoodbornePathogensNaturalToxins/BadBugBook/default.htm

A3: It always depends on what, exactly you are looking for, but here are a few of my favorite websites:

http://www.doctorfungus.org/
http://www.bacterio.cict.fr/index.html
http://www.textbookofbacteriology.net/index.html

They may not always have exactly what you are looking for, but I can usually find something to point me in the right direction.

A4: Files are stripped from circulated messages as a matter of security …

oos form

Hi I`m updating my OOS SOP and I need a model of a form where I could report an microbiological OOS result (Sterility test, Microbial limit test, endotoxin test, B12 assay, Antibiotic validation), please If you could help me I'll appreciate it.

I need to know how you manage the environmental, personnel analysis and water OOS results, like a deviation or no conformance result?

A1: I have 3 available forms (created by me) for OOS investigations:
EM/Personnel, Sterility Testing and Media Fills. If you would like them, please write to me directly at [log in to unmask]

Thanks,
Randy Hutt, Ph.D

A2: Your question is pretty complex as the "OOS" really does not apply to microbiology (reference footnote 3 of the FDA guidance). Part of the problem is that we must first confirm the validity of the lab results before we can devote the effort to a full blown corporate investigation. It is kind of embarrassing to get everyone upset and then determine that an error in the lab is to blame. Better to do the lab investigation first, before declaring an OOS. On the other hand, the Agencies (US and Euro) are becoming increasingly leery of immediately blaming the lab and retesting the sample (a.k.a. "testing to compliance").

If the lab data hold up, then is time to go the larger OOS investigation route.

Couple of thing that might help -

A short monograph on microbiological investigations can be found at http://microbiol.microbiologynetwork.net/resources/monographswhite-papers/investigation-of-microbiological-data-deviations/

Sterility testing for Injection

Regarding sterility testing for injection type product, is it appropriate to do Total Count instead. The result of Total count is less than 1 cfu/ml. Is it acceptable for this type of product? What else can be done?

A1: A sterile injectable is likely to be covered by pharmacopoeial general monographs. One of the requirements is that the product complies with the test for sterility. If this is not included in the product release specification then it is probable that the regulatory agency to whom the application is submitted will require that the test is added.

If you wish to use an alternative test then this will need to be agreed in advance by the relevant agencies. This will undoubtedly require considerable validation data to be submitted. In any case the chances are that the specification will still need to state that the product will comply with the test for sterility if tested.

A2: NO-- "Sterile" for an injectable product means no growth, zero, zip, nada, and the testing for sterility is very clearly specified in all regulations worldwide.

A3: You have to do validated sterility test as per USP <71>

Best Practice in release testing of Isopropyl Alcohol

I have a question in relation to best practice in release testing of IPA for use in production areas.

At our facility we purchase pre-sterilized IPA trigger spray bottles from a well known manufacturer.

As part of release of these to production departments we perform on-site sterility and endotoxin testing of IPA samples and review the manufactures certificates of analysis for each lot delivered.

Does this represent best practice; is there more we could be doing or is this over-kill?

A1: I assume that the 70% IPA is irradiated.

If so it would be subject to parametric release using radiation dosimeters and not sterility testing.

Also bacterial endotoxin testing is not a critical for quality attribute for a disinfectant. What is the mechanism that endotoxin would contaminated a product?

If you are concerned you can determine if the IPA is formulated with WFI.

A2: Over-kill in my opinion. Instead,
1. Purchase from reputed source
2. Audit the supplier for quality (site audit).

A3: I think it is overkill to repeat sterility and endotoxin. I assume the product is gamma irradiated and the supplier provides certificates of irradiation, endotoxin and sterility. Irradiation is the key - The irradiation dose should be validated to provide an SAL of 10 to the minus 6. This is achieved by appropriate studies and documentation (VDMax or Method 1) to set the irradiation dose (usually 25kGy) that gives you your

desired SAL. Also there should be further validation work by the irradiation service provider on dosimetry (to devise a stacking plan and exposure time for each batch to ensure each bottle in the batch receives a minimum irradiation dose to achieve the desired SAL). Routinely then each batch is monitored by dosimetry and then issued with an irradiation certificate. This is the most vital piece of documentation as it proves the batch received the minimum required dose and can be deemed to be sterile or at least have a statistical failure rate potential of 1 in 1000,000.

Sterility testing by your supplier or by you is not very meaningful. Many will explain better than I the shortfalls of sterility testing (trying to spot one contaminated bottle in a large batch of thousands of bottles is very difficult). However, most like the comfort of a sterility certificate for documentation and release purposes.

I think its more important to have fully audited your suppliers manufacturing facility and irradiation service provider. Also to fully assess the irradiation study reports and satisfy yourselves that the work was carried out to a high standard and then make sure an irradiation cert is issued for each batch. If you get a sterility cert too all the better. But dont repeat sterility for each batch its costly and time consuming.

Endotoxin - if you audit your supplier and they have a validated WFI manufacturing plant that is well maintained and they issue certs for endotoxin below 0.25 EU/ml from a recognised laboratory who validated the test for alcohol and water blends, again I don't see the need to re-test.

Alternate test for TOC analysis

Can you suggest an alternate method for TOC analysis with pharmacopoeial recommendation?

Can the oxidizable substances be a substitute for TOC analysis?

A1: Replacing a TOC testing with oxidizable substances test is like replacing a laptop with typewriter.

A2: During Oxidizable substances test, if volume of sample (Water) taken is 100 mL and volume of 0.02 M potassium permanganate added is 0.2 mL, then it is corresponds to limit 0.5ppm (500ppb) of TOC.

The TOC limit is calculated as follows, 0.2 x 0.02 x 0.012 x 1000000 divided by 100 = 0.48 ppm (Rounded to 0.5ppm)

Where, 0.2 = Volume of 0.02 M potassium permanganate 0.02 = Normality of 0.02 M potassium permanganate. 0.012 = Factor calculated as carbon (M.Wt=12)/1000. 100 = Volume of sample taken 1000000 = Factor to report the result in ppm.

As per above calculation data, it may be concluded that Oxidizable substances test as mentioned above is equivalent to TOC limit 0.5ppm.

Further confirmation of above, for Oxidizable substances in the Water for Hemodialysis USP monograph, recommended alternative method is TOC <643> .

I want to know whether this concept applicable for other material like Purified water, WFI, Pure Steam…

A3: yes this is ok but this results is not accurate

A4: Yes. Oxidizable substance is the alternative of TOC. It is in IP.

A5: The Oxidizable Substances test is a very poor alternative to the TOC test.

One of the most important reasons is that the OS test is selective in which organics it reacts with. If the organic molecule is "complex" with long carbon chains and double covalent bonds, the oxidation potential and quantity of permanganate in the OS test has no chance of cleaving all those bonds. The TOC test, on

the other hand, reacts with all carbon molecules in even the most complex organic molecule and converts them to CO_2. So there really isn't a good comparison of limits between the two tests. There are perhaps some very simple organic compounds like glucose that the OS test can give a positive test at levels of 500ppb carbon, but most organic contaminants typically found in water are only partially reactive and will only give a positive OS test at ppm levels.

Frankly, the only reason that TOC hasn't replaced ALL uses of the OS test for pharmacopeial waters is because the organic levels are so high by TOC test results (but still passing by the OS test!), that USP has not been able to establish an appropriate TOC limit for those waters (typically sterile packaged waters that are full of organics that have leached from the packaging). However, a TOC limit is indeed coming.

So please do not consider reverting to the archaic OS test as a substitute for the TOC test. You will do yourself a disfavor, and for USP PW, WFI, Pure Steam, and even Water for Hemodialysis (for which the OS test is being deleted and replaced with TOC), you will be out of compliance!

A6: I would have extreme caution at the use of the oxidisable substances test as an alternate to TOC. The IP is not the primary source of reference in many countries.

A7: I can confirm this experientially. When TOC was first appearing in the pharmacopeia I was testing water from clean steam using both methods. The water always failed TOC but passed OS with no problems. The problem was eventually traced to the plastic hoses that were being used to sample the steam.

A8: Yes, it's an alternate method of TOC as per pharmacopeia but oxidisable substance test is qualitative test with certain limit and TOC quantitative test or more precise.

It's declared in USP and EP and point out as:-

As per USP-33 volume-1 General chapter 1231 (water for pharmaceutical) paragraph Chemical considerations "The TOC test replaced the test for oxidisable substance that primarily targeted organic contaminants ".

As per EP 6.0-volume 2 Monograph, 18.02 water purified "In addition, the test for total organic carbon with a limit of (0.5mg/l) or alternatively the test for oxidisable substance is carried out”.

A9: I have read with interest the opinions on TOC. This one was just for fun.

My concern rests in a lower value that can obtained through a modification of the procedure, whereby the oxidizible substance color is measured by UV/Vis and a more concentrated test solution can be made to enhance the color intensity.

Such a method would require validation, of course, but I would suspect that such a method will yield at least 50 ppb levels and hopefully lower TOC levels as we obtained in our lab through rapid experimentation to verify this opportunity. Our internal water level was 35 ppb, so we were spiking to a higher concentration and verifying a spike recovery.

Warning: time does cause greater color intensity, so a time limit for measurement must be established.

Antiseptic Technique Training Aids

I was wondering if anyone knew of an available antiseptic technique training aid such as an online presentation, DVD, power point presentation, etc. for purchase? I am looking to use this in the laboratory as a training aid for new hires and refresher courses.

100 CFU and Growth Promotion

Does growth promotion need to be performed with less than 100 CFU, or would streaking from a grown culture be acceptable?

When doing the growth promotion, aren't we just looking for the growth characteristics (say yellow colonies with yellow zones on Mannitol Salt agar) rather then yes, <100CFU will grow up on it?

A1: The idea of doing growth promotion test is two fold:

1. whether the media in question is able to support the growth of organisms when less in quantity say 10-100 cfu

2. whether media in question gives characteristic growth of culture where a typical growth is seen or not. hence both is required to qualify the media.

bioburden limit

I am working in Radiopharmaceutical company which produces sterile empty vials and lyophilized product.

All of our product are terminally sterilized.

We do In process bioburden testing for all our product ,and the Specification is 1CFU /Vial , and i think is is too low ,Can some 1 provide the refernce to set the specification of Bio burden test?

A1: To some extent the answer depends on the sterilisation cycle that you use and the heat resistance of the organisms that you are recovering.

A2: It depends on your sterilization method (and how it was validated) and also your product.
Typical sterilization method would allow you higher bioburden levels (i.e.: 10^6, 10^3..), but of course you do not want your Bioburden to reach that high. That's why you set your alert/action limits at much lower level (depending on your historical data).

A3: Still i have one more confusion that As we test In process bio burden (Before Sterilization) Should i consider my product's limit according to the Non sterile product's limit Or Sterile .(BP provide different limits for sterile and Non Sterile).

A4: The answer should be obvious - is your product sterile or not?

Malachite Green

I am looking to order Malachite Green as a primary stain of spore forming gram positive rod bacteria. In the past, I have typically ordered this stain through PML now bioMerieux and our last bottle's expiration date is approaching. The product came in a 250mL bottle with the catalog number R5875. The bioMerieux website shows the product listed but upon trying to order it I was told it was discontinued. I looked into a few other vendors without any luck. The Sigma-Aldrich website showed the product as unavailable in the United States. However, I did noticed some vendors have Malachite Green Oxalate available in the form of a crystalline solid.

I'm curious what other labs are using for spore staining? Also, if anyone knows why this product has been discontinued and where it can be purchased as a liquid it would be very helpful.

A1: I'm not sure if this is any good but we currently order ours through http://www.readyreagents.co.uk/.

A2: My lab experienced the exact same problem. I wish I could point you in the right direction for where you can acquire the crystalline solid, but one of our Senior Analysts recently decided that we would switch to Ethanol Shock tests to distinguish between spore-forming and non-spore-forming Gram positive rods. I

can tell you however that I found the crystalline solid very difficult to work with. It did not enter solution very readily, even when stirring it with a stir bar on a hot plate. Sizable "chunks" of Malachite Green would appear on the slide under the microscope as well. It was likely discontinued due to it being a significant carcinogen (Class II health hazard). Another issue was that we needed to plate our own control sample on the slide, since we could not acquire pre-made control slides for this test.

I have tried to do some searching online but cannot find a description of the Ethanol Shock test for you to read. In summary, the isolate is grown out in TSB, and mixed with 95% Ethanol for a short time. The Ethanol-bacteria suspension is then swabbed onto half a plate, with the un-shocked organism swabbed on the other half as a control. If growth occurs on both halves, the organism was a spore-former as it needed to form spores to survive the ethanol. This technique was unknown to me until our Senior Analyst introduced me to it. If you would like more detail I can provide it, however as I said before I have not found this method in any scientific literature so I can't reliably vouch for its effectiveness. Perhaps another Forum Member can elaborate or help the two of us out.

A3: Engscientific sells Aqueous Malachite Green (5%), Product #6798:
http://www.engscientific.com/micro.html

A4: You can dissolve 5 grams of Malachite Green Oxalate in 100 ml of distilled water for preparing your Malachite Green Solution to stain spores. Besides Sigma, you can order Malachite Green Oxalate from Fisher Scientific.

A5: You may want to make your malachite green to do your stains or find another supplier.

A6: I'm surprised you were able to obtain malachite green in the liquid form. We have always used the powder and then prepared our own solution (5%). Fisher seems to sell a Malachite Green solution (CAT #50-720-427). Try and see if they still carry it.

A7: I checked this website and found an 8 oz bottle that might suite your needs.

www.hardydiagnostics.com
Malachite Green, 8 ounces, by Medical Chemical (MCC) Hardy Diagnostic product code 657A8

A8: There are several options open to you, all of which I've successfully used over the last 30 years:

1. To confirm spores, perform a heat shock (80 C for 10 minutes in a SCDB). Be sure the media is at temp BEFORE inoculating. Remove and cool in an ice bath. Then incubate at the microbe's optimum temp for 5 to 7 days. The spore formers will grow and the vegetative bacteria will be dead.

2. POPPING TEST. Immerse suspect culture in an acidic oxidizer (e.g. 0.1% $KMnO_4$ in 0.3 N HNO_3). The spore cortex ruptures and the cellular milieu spills out for staining with basic dyes. Mount a dried film of the suspected spores on as a dried film on a slide; expose to the reagent for 10 - 20 minutes; observe under oil immersion without staining or with staining.

3. Negative Staining. Use 7% (w/v) nigrosin and observe for highly refractile elliptical or spherical bodies.

4. Dorner Spore Stain. Heat fix the suspension on a slide and cover with thin filter paper cut to fit; saturate with carbolfuchsin and steam for 10 min.; remove paper and decolorize with acid-alcohol for 1 min. and rinse with tap water; blot dry; place a drop of nigrosin on the smear and cover with another slide; slowly draw the two apart to form a thin film; cover with a cover slip or dry; observe. The endospore is red. (This is also called "Flemming's Stain").

5. Alternate Dorner Spore Stain. In a test tube mix an aqueous suspension of the culture with an equal volume of carbolfucshin; heat in a boiling water bath for 10 min.; Mix 1 loopful of 7% nigrosin with 1 loopful of the stained suspension on a glass slide; mix and spread until a thin film forms and dries; examine.

Needless to say, do all these stains with gloves on if you have any life outside of the lab!!!

A9: You have described a very ancient technique in the world of endospores. It is simple and to the point. Most of the time when one works with endospores heat shocking is performed. It is interesting to find that the heat shocked counts can many times be higher than the non-heat shocked. One is to take heat shocked counts as CFUs arising from bacterial endospores as you have the spores exposed to boiling water for 15 minutes. The non-heat shocked counts should have colonies arising from vegetative cells as well as germinated endospores.

My first job in industry was making the endospores that went into the biological indicators marketed by AMSCO during the golden days of those BIs. I also did a good amount of research on the endospores.

A10: To avoid the Sizable "chunks" of Malachite Green, just filter the stain. You do this using a flask and a porcelain funnel lined with ordinary filter paper then apply pressure with a vacuum pump. The preparation and filtering could be messy...

A11: Honestly, I have usually been able to confirm spores in culture with just a simple gram stain and a good microscope. A polarizing filter is very helpful.

A12: Remel sells a pre-made solution.

A13: A simple phase contrast microscopic observation of wet mounts at high magnification [40X Phase 2 or just 100X oil immersion lens observation] has enough resolution to reveal spores. Please see these spore picture of a simple wet mount of a spore forming culture taken for the website photography: http://www.sivams.com/photogallery# [Image 11].

A14: Himedia also supplies malachite green as solid and liquid as well.

A15: After college, I had not gotten my lab coat colorful ever again, To visualize spores, a wet mount under a phase microscope is what I have used. Our microscope had some green filters that made it easy on the eyes to see the wet mounts and marvel at the beautiful and rather large endospores seen. However, if you do not have a phase contrast microscope then it is more economical to use Malachite Green or even a Gram stain as someone mentioned previously.

Is kelbsiella pneumoniae really pathogenic in a gastric scenario?

FDA Bad Bug Book would have us believe that K. pneumoniae is an "emerging food borne pathogen"....really? where are the references supporting this?
The only ones I can find implicating KP in gastric activity clearly suggest it has assimilated virulence factors from something far more nasty, and that since it is then excreted by the patient with said virulence, it can be assumed to have contributed to that patients symptoms... and indeed the FDA BBB does give this qualifier. How then can this scenario be food borne? It only happens once inside an already infected person.

I know of an interested third party who are very concerned that low levels of KP sporadically appearing throughout a batch of non-sterile complimentary are going to cause massive outbreaks of gastro, and the FDA BBB is one of the sources of their concern.

We should all be aware of the risk of over-reacting when applying the Objectionable Organisms concept, and when one of the principle reference sources gives unnecessarily scary data we are going to end up with sterile non-sterile products, and/or very affluent consultants...

Don't be afraid to question "authoritative" sources; emperors with no clothes on, in ivory towers, are there for the taking!

A1: In a previous life I worked for a decade at a large medical reference laboratory in the microbiology department and had the pleasure of looking at large numbers of stool cultures. We never considered the Kelbsiella pneumonia isolated from stool as pathogenic. It was treated as a pathogen when isolated from sputum, wounds, and the genitourinary tract in suitable numbers.

A2: Unfortunately, organisms change and adapt to survive. That is the problem with Klebsiella pneumoniae. Multi-drug resistant Gram-negative organisms (especially Klebsiella pneumoniae) that harbor extended-spectrum beta-lactamases (ESBLs) are increasing in frequency in hospitals, nursing homes and other community settings in the United States. The organisms produce beta-lactamase enzymes, which are able to hydrolyse beta-lactam based antibiotics such as penicillins, cephalosporins and carbapenems. Resistance to carbapenems, a class of broadspectrum antimicrobial agents reserved for treating infections caused by gram-negative organisms resistant to most other agents, is a challenge to clinical microbiology laboratories. The most common strain isolated in the US is the carbapenem-resistant Klebsiella pneumoniae (CRKP). CRKP causes serious healthcare-related infections and often occurs in patients who are treated for chronic conditions or patients who have prolonged hospital stays. Carbapenemases are enzymes located on a plasmid which can be found in carbapenem-resistant Enterobacteriaceae (CRE) like CRKP. The enzyme, the Klebsiella pneumoniae carbapenemase (KPC), is mediated by the blaKPC gene. Currently, it is the most common carbapenemase in Enterobacteriaceae isolated in the United States. It has been reported on plasmids with other β-lactamases [including the extended spectrum β-lactamase (ESBL)] as well as aminoglycoside and fluoroquinolone resistance.

Beta-lactamase producing organisms frequently colonize the lower gastrointestinal tract, where the genes encoding the resistance enzymes may be easily transferred and maintained in the host flora without causing an infection. ESBL-producing organisms may also transfer their plasmid-encoded genes to nosocomial pathogens as well. Today, Klebsiella pneumoniae can be a very bad bug!

A3: The CDC has some very good information on Klebsiella. http://www.cdc.gov/ncidod/dhqp/ar_kp_about.html
There are some good articles on this site as well: http://emedicine.medscape.com/article/219907-overview
One CDC article (available on their website) states: "K. pneumoniae has been a recognized pulmonary pathogen since its discovery >100 years ago." (Emerging Infectious Diseases * Vol. 8, No. 2, February 2002)

It is very important to assess the route of infection when determining whether a microorganism is objectionable in non-sterile pharmaceutical products. Other factors to consider are the intended patient population, infective dose/number of microorganisms present, the ability of the organism to damage or spoil the product or container, the dosage form of the product (relates back to the route of infection), and the ability of the microorganism to proliferate or sustain itself in the product. The determination of whether an organism is objectionable should be based on a risk-assessment approach taking into account the elements defined in the USP.

A4: Klebsiella could be an objectionable organism because it is gram negative and is an agent of nosocomial pneumonia. I have never heard of it as a pulmonary pathogen. To me it will always be objectionable and always has been.

A5: K. pneumoniae can cause pneumonia but more commonly causes hospital-associated urinary tract and wound infections especially with patients with immuno-suppression.

An important question is whether a contaminated product administrated via one route of infection can cause infection at another site. A recent publication demonstrated that E. coli-contaminated chicken can lead to urinary tract infection via fecal material.

Could aspriation of K. pneumoniae cause pneumonia? Some 15% of community-based pneumonia is aspiration pneumonia. Risk factors for aspiration pneumonia include stoke, drug abuse, coma and gastro-osephageal reflux.

A6: I refer you to the CDC article below which specifically addresses Klebsiella pneumoniae as a "pulmonary pathogen" (thus the name pneumoniae). It is very important to assess any microorganism found in terms of its route of infection and your patient population. For example, Bacillus cereus can be an objectionable microorganism when it reaches a population density adequate to initiate toxin production. In oral products, then, high numbers of B.cereus would be considered to objectionable. In a topical product, however, B. cereus would no longer have a suitable route of infection to cause disease. Would you consider Klebsiella pneumoniae objectionable in a topical product? Microorganisms found in products should be assessed using the guidance in the USP. A science-based risk assessment which examines all the factors listed below is, to me, the only way to properly assess whether an organism is objectionable in your products.

A7: In a former life - within the Nutritionals sector a very high percentage (probably there is an official estimate but it escapes me) of K.pnemoniae isolates were considered faecal coliforms / thermotolerant coliforms (perhaps one might consider it an outdated term but its still used I believe) as they were able to grow at 44.5oC and were picked up on the E.coli selective test method.
The fact that they were not E.coli was unimportant, they were faecal coliforms and food products at least in this sector were required to have an absence of Faecal Coliform per standard test quantity, while a limit of less than 10 cfu / g was acceptable for non-faecal coliforms (transient gut colonisers or environmental contaminants form vegetable matter raw ingredients etc, or those and not specifically associated with faecal contamination).

So while some such as Klebsiella oxytoca might be considered objectionable depending on potential dosage form, route of administration etc K.pneumoniae (at least thermotolerant strains) irrespective of its name or the potential to cause nosocomial infection in certain high risk groups, would be by rote considered objectionable as they are indicative of adulterated product and poor hygienic practice at some stage of the production or handling chain. The requirements above would have been derived from the FDA BAM circa 92 - 96. It has since morphed into the Bad Bug Book so I dont know if the approach has changed.

A8: As Tony suggested 15% of community acquired pneumonia is due to aspiration pneumonia. This number doubles in hospitalized patients. A significant portion (not majority)of these are due to KP. Along with oropharengial organisms, any viable organism in the aspirated material is a potential causative agent. As suggested, colonization at any site can act as a nidus and lead by bacterial tropism to establishment of infection at a more characteristic site. Regardless of the point of entry, some organisms end up at a specific loci. Bacterial translocation from the gut to other parts as well as to the blood stream by way of mesenteric lymph nodes have been described for KP and other organisms. Indeed the gastrointestinal system is the "usual suspect" in any major infective process that is caused by gram negative organism. However these are not FDA's rationale for inclusion of KP in the list. In the FDA BBB, KP is specifically associated with acute and chronic Gastroenteritis. But even FDA did not elevate KP to the status of the more infamous villains that enjoy their own subsection in the book. It rather lumps KP with others in a not so flattering category of "miscellaneous" perhaps more for the sake of "completeness" rather than as an acknowledgment of a serious public health risk.

Most of the data that associate KP with acute or chronic gastroenteritis come form the developing world where in most instances Gastroenteritis is the #1 cause of morbidity and mortality. Though not many, there are a number of studies that suggest the following.

- “Enterotoxigenic" strains of KP have been shown to cause acute gastroenteritis in young children (Asia, Africa).
KP gastroenteritis has also been identified superimposed with rota virus enteritis.

- KP is a major contaminant of food items particularly meat, milk and leafy vegetables. Some studies have presented evidence that a horizontal transmission in food items (meat) exists and can even lead to a formation of antibiotic resistant biofilms contributing to a progressive increase in antibiotic resistance of KP in the community.
- Pyogenic liver abscess due to KP (K1 and K2 serotypes) are not uncommon in diabetics. The portal circulation by linking the gut with the liver may provide a way for the spread of infection.

- In isolated adult cases, KP gastroenteritis has be shown to be present but remain masked by more aggressive etiologic agents.
- As FDA BBB suggests, the infectious dose is not known and is probably high as only immunocompromised or debilitated patients are mainly affected.

Having said this, in my opinion, the epidemiology does not seem to warrant the conservative use of FDA's inclusion of KP as food born bad bug as a rejection criteria. A mere presence of limited number of organisms in an oral preparation or food should not be a cause for alarm. A heavy/gross contamination however may be a cause for one. Particularly if the device, or the product/food is intended for use by a population at risk eg. NG feeding of recumbent patient.

A9: Note of clarification: I am fully conversant with all the usual pathogenicity of K. pneumoniae; urinary, lung and catheters...the question is solely whether this species can be also be classed as a food borne pathogen, that is a realistic risk of gastric upsets...no-one is seriously at risk of snorting the contents of a capsule or grinding up a tablet and sniffing that, (or rubbing tablets on open wounds, or using them as pessaries), and we are not required to seriously consider those scenarios in our risk assessment processes.
Furthermore, I am fully conversant with all the considerations for Objectionable assessments; the product is stable with very low water activity, levels are at the limit of detection, and often only by enrichment and there is no risk of degradation during shelf life.

I reiterate: is KP really capable of causing gastro if ingested in a capsule? FDA BBB says Klebsiella spp., Citrobacter spp., Serratia spp., etc are all "emerging food borne pathogens" in this scenario...forget KP's "normal" pathogenicity for a moment...where is the evidence that this bug can give you diarrhea if you eat <10 cfu/gm of it?

A10: One must consider the source of Gram negatives. Who's to say that a distant cousin, E coli, possiblly from the same source is not lingering in the mix. Just because you didn't find in the lab doesn't mean it isn't there.

A11: This has nothing to do with answering your question, however to add to your Kp interest, this lovely little article came across my desk this morning from my R&D department. It's worth at least quick look...

http://www.usatoday.com/yourlife/health/medical/2010-09-17-1Asuperbug17_ST_N.htm?csp=hf

A12: Your link doesn't appear to be working; any chance you could send a copy of the article please?

A13: Try searching "drug resistant superbug usa today" in Google. I found it there. I guess it doesn't open using the link because it starts with a cell phones advertisement.

A14: Now we enter into the concept of Indicator Organisms. KP is not an indicator of Faecal contamination due to its widespread presence in the environment as well as (sometimes) being associated with the contents of the human gut. It is arguably longer lived in the environment than either E. coli or its indicated pathogens. E. coli is used as an indicator due to its frequent (dedicated?) association with gut contents and also its lifespan outside the gut after excretion is considered similar to other, pathogenic, members of the gut population that are more difficult to test for (e.g. viruses). If we try to double guess the presence of E. coli , and therefore these other nasties for which it indicates, by the presence of KP or other, non-pathogenic, non-indicating species, we'll be jumping at shadows forever.

We tested for E. coli by enrichment method, per BP2009 harmonised, and satisfied the criterion that it should be Not Detected by that method. We are permitted <100 cfu/gm BTGN by MPN but if , as you now suggest, many, or all of those BTGNs are now indicating possible E. coli then the spec would have to be tightened to <10 BTGNs...and even then, it's a softer test than ND in 1 gm by enrichment. Not required, not practical, and poor science.

A15: The link goes onto the line below, you just have to copy the whole link and paste it into your browser!

Temperature Mapping of incubation Room

I learn an issue regarding temperature mapping & need your opinion please. In a temperature mapping of Media filled vials incubation room condition 20 to 25°C, it was observed that temperature at many occasion (~10-15% of total data at different time point and not continuous) gone below the lower limit i.e. up to 18.5°C.

When MKT is calculated, it was just above lower limit i.e. 20.25°C.

1. Can temperature mapping study of media filled vial incubation room be accepted in above case?

2. Is it acceptable to justify the study with MKT, my concern is that it is not simply storage, but it is facilitating growth of Fungi; if any?

3. If accepted, how can be explain the impact on pass results i.e. it is true results and is not affected by temperature excursion?

4. Can the same situation be accepted for other condition room i.e. 30-35°C?

Microbial Identification: VITEK 2 versus BIOLOG

I would like to know your opinion about microbial identification technology. We have been using VITEK 1 for the last 6 years. Now we have to decide if to upgrade to VITEK 2 or to purchase a BIOLOG system. If anyone has experience with both systems I would like to hear your advice.

A1: Really prefer the BioLog system.. In my opinion, easier to use or user friendly, more so for BioLog than Vitek. On some spore formers, BioLog had high ID where Vitek failed to differentiate enough to give ID. Possibly data bases have improved.

A2: Please take a look at the following link:
http://www.microbiologyforum.org/PMFNews/PMFNews.06.04.99.pdf

From our own PMF Newsletter (in 1999), Ed Balkovic from Genzyme Corp. does a great job comparing and contrasting the two. Hopefully that

A3: You can try MIDI Sherlock system in which ID of bacteria is based is based on Fatty acids.

A4: Both system are good, but i give more points to vitek 2 compared to Biolog... Even though biolog is also having a good microorganisms library for reference but you have to do more manipulations than in vitek 2. I recommend Vitek 2.

A5: How does Vitek compare to Biolog in identifying the environmental isolates and clinical isolates.

A6: I have used both systems in the past and they are about the same. Now we had updated the new Biolog that is OmniLog and we have the VITEK2 and again they both work ok. I really like the new VITEK2 (more user friendly than VITEK1) and the OmniLog/Biolog has a bigger database that included some yeast and mold.

A7: We haven't used VITEK, i hope it very good id system too. Since last year end we have Biolog system and we are very satisfied with it. We haven't got any problem with identification of monitoring isolates, purified water flora etc. Very big advantage - possibility to identify BI (we tried two different). I think that yeast and fungi identification is very useful too.

And please take into account that comparison with VITEK in 1999 was made with old Biolog kits and software as i could understand. Now Biolog have GEN III, which is really better than previous version (we tried both).

A8: to compare different RMM systems, you can have a look at the website of ECA's RMM Working Group. In the member area, you will find a database with information about several systems and a best practice paper from Leo Pharmaceutical about Vitek2. The registration to get into the member area is without any fee.
http://www.gmp-compliance.org/eca_rmm_membership.html

A9: Thought I'd pass on this event I came across on Rapid Microbiological Methods: Critical Aspects and Recommendations for a Successful Implementation. Here's the link for more information www.gbprinc.com

A10: if we want to talk about RMM events, another possibility to get in contact with suppliers and users of different available RMM System and discuss with them you can find at www.microbiology-conference.org I think, amongst others, one interesting topic there will be the information about the ECA's survey about RMM and Chapter 5.1.6 of the EP. in this survey, 76 participants gave information about their RMM applications, approvals and comments to the EP chapter.
Last year there were at this event 15 suppliers and nearly 100 users of the different systems.

A11: I have used both systems and they work great. I would recommend Biolog/OmniLog because the database is bigger than the VITEK2 because includes yeast and mold. If you do not need yeast and mold VITEK2 would work fine.

TNTC result & Sterility for liquid sugar

I would appreciate if anybody can share his knowledge and comments with me about following subjects,

1-Please let me know if you get a TNTC result for your total count and Yeast&Mold after first dilution 1/10 what you would do?Considering you have a natural product.Should we do more dilution and if yes, up to what dilution.Please note that in one of the cases we did dilution up to 1/1000 and still got TNTC.How do you interpret this result.In USP 2021 it say if necessary dilute more.anybody can clarify this please.

2- I want to do sterility test (USP 71) for liquid sugar.This product goes to which category and what method is the best?

A1: When testing for natural (or any products) especially without any historical data. You would generally dilute in accordance with the specification limit. If your product has a specification limit of 10^5 then you would dilute to 10^-4, or if your product had the limit of 10^2 (as most things in the harmonised pharmacopeia do) then you would test to 10^-1.

The general acceptable limits for accurate counts on an agar plate are 30-300. So if your product has greater than 300 colonies on its agar plate test at the specified dilution then not only does it fail its micro but does so by greater than a factor of 10.

Having said that, we do test natural products here and there is a variation between a lot of them where one type can return zeroes and another be approaching acceptable limits regularly.

What you must firstly determine is whether or not you are looking at sporulation on plates (in which case you need to read your plates on a daily basis from initial test point) and secondly, did you get a CofA from the manufacturer, although in my experience these are produced to cover greater than a single batch and can be misleading.

I would say dilute away and build up an idea of what kind of bacterial loads this product generally holds, but if you are consistently reaching or even surpassing specification limits then you may have a problem with your supplier.

A2: To get rid of TNTC , making higher dilutions is only the solution, if it is giving TNTC in 1:1000 then you dilute it further till you get countable colonies up to 300. Definitely you will get answer to your question, use 0.85 % saline or phosphate buffer for making dilutions and count the colonies on 24th hr of incubation don't wait for 72 hrs. however final results will be of 72 hrs.I am doing it with natural samples like soil etc.

A3: The dilution you do will help in the enumeration of the cfu/ml and this will be used to calculate the possible cfu/l or cfu/g in the original sample by multiplying with the dilution factor. what ever dilution factor you have used the cfu will have to be checked with the standard specification for the sample in question .Thus rejecting or accepting depending on the specification. When it is TNTC at 1/1000 it is obvious that the sample will fail for a standard specification of NMT1000 cfu/ml.

For liquid sugar prepared in-house for production use , it will be categorised as an intermediate product, so the same spec for product will apply.

A4: I am showing below some results:

1. 10,000 dilution of a sample gives 1 colony on one plate and 2 colonies on the duplicate plate. what should I interpret should I average them or take the greater one?
2. Same case in one plate i got 4 and duplicate gives 10 ? what is the criteria for averaging the results?
3. In an other case, one plate shows 1 colony and other plate gives zero. what should I do?

A5: There is a white paper I posted that might be of help at http://microbiol.microbiologynetwork.net/resources/monographswhite-papers/co unting-colonies/.

Basically, you will need to develop an SOP on how to handle these plate counting issues. The problem isn't so much how you decide to handle them as it is that you must do it the same way each time. There are a variety of documents that provide guidance (see white paper above for some of the major ones) but in the end it depends on your SOP and the rationale used to determine the "company approach".

As an aside, while preparing an SOP on "counting colonies" you might also want to put one together on lab math issues - how to round, significant figures, log conversions, etc. This is another area for mischief in the PET.

VRBGA

Anybody has an explanation regarding VRBGA (used in USP 62 for Enterobacter) & VRBGA+Lactose (used in USP 2022 for Enterobacter).What does the additional lactose do in the media? What quantity of Lactose and what kind of lactose should be added to media? Is it justifiable if we use VRBGA instead of VRBGA +Lactose? if yes how we could justify it?

Antibiotics microbial assays: Neomycin interferes in Polymyxin

How to make the Antibiotics microbial assays for Polymyxin in a otic product that in addition has Neomycin and that interferes in cylinder plate method?

A1: You need to add an appropriate quantity of Neomycin standard (compensating standard)in your final Polymyxin standard preparation to equal the quantity of Neomycin in your final product dilution.

Compare the assay results with and without the addition of Neomycin in the standard preparation.

If there is still some difference in the assayed potency after addition of the compensating standard, you will need to evaluate the other ingredients in the otic formulation to find out whether they are exerting an indirect effect on the assay culture.

A2: Neomycin Sulphate and Polymixin Beta Sulphate both are water soluble material. But in microbial assay those materials can not interfere because M. assay of Neomycin Sulphate is carried out against Bacillus Pumilus and Polymixin B Sulphate is carried out against Bordetella bronchiseptica. I performed these assay for last 10 years, I did not find any interference between those. So you can perform M. assay for those materials confidently.

A3: Could i use other organism to make Antibiotics microbial assays for Polymyxin in a otic product that in addition has Neomycin and that interferes in cylinder plate method?

Clarification of endotoxin indicator for tunnel reg

The following is the statement picks from USP 32 chapter <85> from the foot note number 2 < page number 93, volume 1> " for a validity of the procedure for inactivating endotoxins , see dry- heat sterilization under sterlization and sterility assurance of compendial articles 1211 . Use an LAL reagent having a sensitivity of not less than 0.15 endotoxin unit per ml.". Based on the above statement , shall we use the 0.125 or 0.06 endotoxin unit per ml sensitivity or we need to use 0.25 endotoxin unit per ml sensitivity lysate for tunnel or DHS validation. Kindly clarify.

A1: If you want to verify depyrogenation through DHS you have to show 3log or 6log (?) reduction (10^3 or 10^6) of endotoxin. In this case maybe you should need enough endotoxin spiked subjects.

A2: Carefully transfer Endotoxin Indicator through Tunnel as same when product containers are passed. Carry out LAL test for that EI for checking of endotoxin reduction. The best result may be come from Kinetic Method for exact Log Reduction. Gel Clot may be applied. If you use Gel Clot, you have to prepare serial dilution up to Lysate Sensitivity.

A3: In response to the two questions:

When the USP states "Using a reagent sensitivity of not less than 0.15 EU/mL...." That means that the sensitivity must be higher greater than or equal to 0.15 EU/mL (i.e. the reagent must be able to detect endotoxin concentrations of 0.15 EU/mL or a lower amount of endotoxin). For example, 0.125 and 0.06 EU/ml are more sensitive than 0.15 EU/mL and will comply with that statement.

USP chapter on sterility <1211> states that a three- log reduction/destruction of endotoxin should be demonstrated. Please tell me if you know of any documents referring to a six log reduction.

ozonisation of microbiology laboratory : an alternative to fumigation with formalin

I have few questions regarding this subject

Can we ozonize our microbiology laboratory and how?
Ozoznization is only applicable for water or air also?
Air purifiers available in market are really an alternative to the fumigation process in pharmaceutical industries and microbiology laboratories?
What should be the validation process in installing an air purifier as an sterilant inside a microbiology laboratory ?

A1: We are currently using PRO-8 Ozone Generator
(http://www.jenesco.com/pro-8-ozone-generator.html)

in small areas including our lab, not as the only practice to clean the entire lab but in addition to our current practices using disinfectants. We use it as preventative maintenance every 3 to 6 months in other areas, it took over a years' worth of data to show that this actually does cut down contamination levels (validation). The link above is to our system which offers a 30 day trial.

As for lab ware... we are currently testing against our normal sterilization methods. We are very pleased with the results in some of our hard to clean pieces.

With other questions and/or demonstrations I would contact the company that supplies the machine.

A2: In most countries there are environmental regulations for the release of ozone to the atmosphere. Ozonation of closed liquid systems is allowed with ozone destruct meter and in place and properly working. Ozone is not allowed to be used in an open space.

Fumigation or more commonly called sanitization should be a routine in the lab. Alcohol and 5-10% bleach are acceptable, purchasing prepared solutions from Steris is more the routine.

A3: Might look at Chlorine Dioxide gas. We have used tablet generating Cl02 gas in the lab area. We keep the concentration low to avoid metal corrosion. With these tablets, we place a 500ml beaker of tap water on a magnastirrer and add the tablets. Tirn the stirrer on and the tablets generate the gas. Two fans in the room (small 8" box fans) help distribute the gas. Very inexpensive and easy for the staff to use. We let the room set for 1 hour of gassing and then another 2 hrs. to dissipate the gas. This is easy enough to be used frequently or when needed. NSF International has published a protocol (In Standard 49)on the use of Cl02 with BSC that could be applied to the process using certified Bacillus atrophaeus spore strips as your biological indicator of efficacy... I can help you with the strips. We get our tablets from Quip Labs in Delaware.

microbiology labs manager

Are you agree? I need your opinion on the following:

New commentary for Second Supplement to the USP 33-NF 28 Reissue, stated in page 6 the following comments on good microbiology best lab practice: Comment Summary #8:

may be demonstrated …understanding." expected that laboratory supervisors and managers have a demonstrated level of competence in microbiology at least as high as those they supervise" notion is unrealistic and inconsistent with the principles of management, and, b) " that microbiology is a scientifically based discipline that deals with biological principles substantially different from those of analytical chemistry and engineering disciplines. Many times it is difficult for individuals without specific microbiological training to make the transition."The commenter recommended re-wording the paragraph "Competency, and deleting the following sentences a) "Further, it issince, according to the commenter, thisIt should be notedResponse:

chapter indicates that) only and not a requirement.

read the full text of this commentary at http://www.usp.org/pdf/EN/USPNF/USP33-NF28SecondSuppCommentary.pdf

A1: With respect I went to the web site you mention (http://www.usp.org/pdf/EN/USPNF/USP33-NF28SecondSuppCommentary.pdf) and offer this edited version of the passage for ease of reading.

As an aside, I believe the committee was correct in failing to incorporate this comment.

<1117> Comment Summary #8: The commenter recommended re-wording the paragraph: "Competency may be demonstrated …understanding.", and deleting the following sentences [Note: reference (a) and (b) below] since, according to the commenter, this notion is unrealistic and inconsistent with the principles of management
a) "Further, it is expected that laboratory supervisors and managers have a demonstrated level of competence in microbiology at least as high as those they supervise" and,

b) "It should be noted that microbiology is a scientifically based discipline that deals with biological principles substantially different from those of analytical chemistry and engineering disciplines. Many times it is difficult for individuals without specific microbiological training to make the transition."

Pre Incubation of sterilized media

i want to known about the preincubation time of the prepared media used for Env. monitoring, ie how much time we have to preincubate the media 24 hrs or 48 hrs, is there any reference guideline plz give the details.

A1: Preincubation time depends on your test if your test is critical then incubates the plates for that time which is required for your test e.g. in your case for environmental monitoring program we incubate the plates for 3days for bacterial count & 5days for yeast& mold count in two different temperatures incubators so we have to incubate the plates for 3days for bacterial count & 5days for yeast& mold count these plates are refereed as negative control for your test Your 2nd question is that any guideline give this reference yes all guidelines gives these instruction that before any test we have to preincubate the media plates how much your test duration because you incubate plates for only 24 hours &48 hours but after this time any abnormality comes in the media the we cannot judge so it is very necessary to preincubate the plates for that much time that your test have.

A2: please try to read ISO/TS 11133-2 2003. There you can find out how to control media.

Norfloxacin tablets

is there someone out there that examines microbiological purity of norfloxacin tablets? How can I neutralize norfloxacin's antimicrobial activity?

I tried the following:
Tablets quickly disintegrate and when the solution stands still for 15 minutes, a supernatant can be seen above the precipitate. If I filter the supernatant (10 ml of it, equaling 1 g of the product), rinse and put the filter on tryptic soy agar, not even one of the test microorganisms grows. If I do the same for the E. coli test, and place the filter in the broth, no growth occurs as well. Too bad. I tried to filter 0.1 g (1 ml of the solution), but B. subtilis still does not grow at all.

What do you think?

Can you suggest a neutraliser?
European pharmacopoeia suggests some neutralisers for certain antimicrobials - but where does norfloxacin go? There is a fluor atom - halogens? Some OH groups, =0 groups, -COOH groups.... I don't know what to take - a mix of almost all suggested inactivators? Will that do the trick?

A1: If you do challenge test don't filtrate the product add 100CFU directly to 10g nor-flox.and 90ml cas-lecithin broth and take 1ml on plate and pour TSA.

A2: I tried the pour-plate method as well - sample was prepared with buffered sodium chloride peptone solution - bacteria showed 0% recovery even at 1:1000 sample dilution.
Was your cas-lecithin broth acc. to the previous version of Ph. Eur., or did you use some other cas-lecithin concentrations?

A3: Can you, suggest me the procedure for Sanitization of the RO membrane?

Name of bacteria

I want to know the name of bacteria which can pass through 0.3 µ HEPA filter.

A1: I speculate it as brevundimonas diminuta.

A2: Psedumomonas diminuta. I think Mycoplasma sp. also can pass 0,3 um filter.

A3: BREVUNDIMONAS diminuta-- they changed the poor bug's handle a while back- is nothing sacred?

A4: I have heard of B.diminuta being used in the HIMA/ASTM bacteria challenge for validating sterilising grade (absolute 0.2micron) membrane filters, but not for HEPA filters. A sterilising grade membrane filter must prevent the passage of all viable cells in a suspension of B.diminuta equivalent to 1 x 10 the 7 cfu per cm2 of membrane surface to pass and be rated as sterilising grade. I am sure if you could find one, a 0.3 micron membrane filter would allow B.diminuta through albeit to a low level but not sure what that would tell you.

HEPA filters are usually depth filter not absolute filters so do not have a pore size rating eg 0.3 micron.

Testing of HEPA filters is achieved by DOP testing.

Sterility test repeat

I Have a problem. Sterility test of an injection turns positive during 13th day of incubation. Please guide me that what should i do? Is it sterile? Or i have to repeat the test. Is it possible that during 13th day of incubation an organism is showing its presence?

A1: If you incubated it for 13 days - you established a de facto expectation that growth could appear on day 13. By your data, it is not sterile.

A2: NO it is not sterile- at least according to the test results. I would perform an investigation to determine the root cause of your positive result. I would review all test materials, training, environment, etc to first rule out any involvement of the testing itself in the positive result.

I am assuming this was a sterility test of production material and an investigation into manufacturing should be performed to ascertain their part in the positive result.

Unless your investigation shows that the positive came from your testing and not the product, you can not repeat the test.

A3: After 13 days positive in sterility test does not mean that your product is failed. Read carefully Interpretation of result of BP or USP or EP. You can repeat this test when the following criteria found in the test :

If your environmental monitoring data of sterility test found error
If positive growth found in Negative Control
If your analyst did not maintain aseptic technique or lack of knowledge on aseptic technique. If match anyone now you can perform repeat test and that case, first test will be canceled, repeat test will be first test. Repeat test is not allow without any justification.

This product must be failed when you did not find the above any error (a to c)

A4: Is there an investigation process in place for sterility positives? If not, there should be.

You will obviously want to check the analyst's training, any EM performed in the isolator, check the isolator for breaches, and at the very least, identify the isolate so that you can find out if it is human flora or an environmental isolate!

It is definitely possible for the organism to not grow out until the 13th day - if it weren't, why would the regulation groups want us to incubate for not less than 14 days?

A5: The product fails the sterility test. It is possible for organisms to grow late in the test period - this is why 14 days is specified.

A6: Better do to laboratory investigation first if there is any lab. error observed then you go for repeat analysis.

A7: This is no doubt a sterility failure.kindly share the growth results of positive and negative controls.

A8: Repeating test is not allowed till you prove its a lab error. Identify the organism to know if it is slowly grow. But 13 days is a long period.

A9: It is not wise decision to tell "failed in sterility test" without any confirmation that microorganism found the test result. That microorganism should be identified for proper investigation.

13 days is not long period because you should be incubated not less than 14 days. So if you find growth at 14 days, that will be definitely under investigation.

Not so easy about Sterility of product.

A10: To clarify - the product failed the first phase of the sterility test. Any growth up to and including day 14 will do this. In specific circumstances the test can be repeated.

A11: The growth with in 14 days is indication of sterility failure and hence you have to investigate what organisms it is and what could be the source of contamination.

A11: Yes i would repeat the test, according to Pharmacopeia any growth observed during 15 days period is taken as failure to comply with specification.

A12: There are certain bacteria like propinibacterium acne which is a slow growing bacteria it takes nearly 10-14 days to grow.

A13: if we get the growth on 14th da also the test may be in valid or the product may be non sterile.

Here i am requesting you please share the organisms that shows growth after 11 - 14 days

A14: Propionibacterium acnes is often the culprit when late incubation positives are observed. I have seen this organism show up after 13 days of incubation as well. Be sure when you try to confirm that you allow sufficient time for the organism to grow. If you are streaking for isolation pour your plates thick so they do not dry out. Incubate one plate aerobically and one anaerobically.

A15: Organisms that take a long time to grow during the sterility test incubation period have probably been damaged at some point in the processing of the product concerned. As far as I am aware there are no specific limitations on which organisms might behave in this way.

Before conducting a retest it will be necessary to justify that the first one is invalid.

A16: The name of microorganism is propinoibacteriun acne.

A17: If the contaminant orgs identified and proved to be nonsporing fast growing orgs, moreover it is highly susceptible to product orgs can we not consider it as a contaminant occurred during test & be given advantage of repeating the test. Of course one should investigate the sterility testing method also.

A18: Methylobacterium radiotolerans and other Methylobacterium species can also show up very late.

A19: I would be cautious with this approach. While I understand that it is possible to suspect lab error as the cause of the sterility test failure, you cannot invalidate the test without clear evidence of this fact.

These conditions are clearly described in the harmonized sterility test (USP <71>, Pharm Eur 2.6.1), and I would also refer you to:

Pharm Eur 5.1.9. Guidelines for Using the Test for Sterility
USP <1117> Microbiological Best Laboratory Practices (current revision at 2010 USP33 Reissue Suppl 2 pp.R1100-R1105)
TGA Guidelines for Sterility Testing of Therapeutic Goods - http://www.tga.gov.au/docs/pdf/sterilit.pdf
PIC/S PI 012-3 Recommendation On Sterility Testing - http://bit.ly/b82sQr
PIC/S PI 014-3 Recommendation Isolators Used for Aseptic Processing and Sterility Testing - http://bit.ly/cCCfL1

Finally, there is a chapter on "Investigations" that discusses this against the general background of Laboratory Investigations in the new publication "Laboratory Design: Establishing the Facility and Management Structure" which I would recommend. More information on this is at http://bit.ly/b5r2ZY

Be very careful with investigations - these are becoming a focal point for GMP audits in recent years.

A20: Clear evidence of lab error means orgs found in the product is same as that found in sterility test personnel results ie. gloves or laboratory environmental data.
However as there are abundant varieties of normal flora of skin/air.It may not be possible to match the same strain found in the product & at the same time on gloves/& in sterility test area..For this reason depending on the characteristics (ie.Susceptibility to the product & nonsporing orgs) of the contaminant & depending on the complying data / trend of environmental data of testing as as well as production area & other parameters, can we not invalidate the sterility test as lab error & repeat the test.

Can matching up to Family/genera/same type of flora can be considered as a clear evidence to invalidate the test as lab error? This may be possible for the most of the times.

A21: The guidance documents are absolutely clear on this point - you must show that it is the same strain in both locations if this is to be the basis for invalidating the test. At this, we are catching a break as logically we cannot argue that isolation of the same strain proves that contamination of the filling equipment (or whatever) CAUSED the product contamination, only that it was CORRELATED with the product contamination.   In any event, if they are different strains you have no argument.

You also mention several other mitigating factors such as the product's preservative system and EM data. The preservative system is a relevant argument as one would expect an organism suspended in the product to die-off if it were shown susceptible to the preservative system. However, we have no evidence what condition the organism might be in or if it might be in a protected location in the container (in the closure system, in a biofilm?).

The second argument you made is irrelevant to this discussion. That your facility was in a state of control (as evidenced, in part, by the EM data) only proves that you were capable of making a sterile product if nothing else goes wrong. It does not provide any evidence that you successfully did make that sterile product.

I honestly think that the best you will be able to salvage out of this is going to be based on the antimicrobial efficacy data. You may be able to argue that it was possibly a lab error, but that the results are inconclusive. Since the data are inconclusive, the lab test stands.

This is, by the way, why it is so critical to have a lab in which you have complete faith. False positive results on QC product release tests become VERY expensive. It is much cheaper to pay for competent work. (not that I fault the lab in this case - the crux of my argument is that there is no good evidence that the lab did anything wrong).

The method described is based on 21 CFR211, USP <1117> PIC/S guidance and years of experience auditing micro labs (internal QC and CRO) for clients as part of qualification and investigations.

A22: It is quite possible for an organism to present itself on the 13th or 14th day of incubation. It may be that the organism was initially damaged and it took some time to repair and produce growth, or it may be that the growth conditions in neither of the sterility test media is optimal for this organism and thus it is struggling to reproduce. In either case the product fails the test and an investigation needs to be performed to determine if the product is non-sterile or if there was an error in the testing of the product that contaminated the test. If it cannot definitively be shown that the problem was with the testing, then product fails the sterility test. (Even if you have a negative result on retest, the sensitivity of the sterility test is so poor that if you are testing a batch that is contaminated at 1/1000, 0.1%, you will pass the sterility test 98% of the time.)

Anaerobic Media Fills

I need some information on anaerobic media fills. Can you address the following questions:

1. Do you perform anaerobic media fills? If yes, how often and for what type of products(nitrogen overlay, other)?

2. If you perform anaerobic fills, was this due to a FDA requirement or your own assessment?

3. Do you also do monitoring of the environment for anaerobes?

PET testing

I have a few questions about Preservative Efficacy Testing I was hoping to get some guidance on. I work with a company that does contract Microbiology testing and we are hoping to get our method for PET testing approved by our local regulatory body. Currently I am setting up the method based on the European Pharma for oral and topical products only.

The method appears straightforward enough it is just the validation I would like some opinions on. Currently for validation I dilute the product to be tested 1:10 in neutralizer (Buffered peptone saline with tween) and 1:100 in neutralizer. Then I take 1ml of the 1:10 dilution mix it with 100ul of 30-300 cfu of one of the microorganisms under investigation (S. aureus, P. aeruginosa, E.coli, C. albicans or A. niger) on an agar plate and pour with the relevant agar (Tryptic soy agar or Sabouraud dextrose agar). The control then is to mix 1 ml of the neutralizer with the same quantity of the microorganism on a plate and pour with agar. The pass criteria is recovery of microorganism with product and neutralizer must be within a factor of 2 of the recovery of microorganism with neutralizer only. If this fails we repeat using the 1;100 dilution of product and then if this fails we use membrane filtration. Am I covering all possible angles here for validation? And are the pass fail limits ok?

I was also wondering has anyone got accreditation or approval for this test and typically what numbers of samples they would test and how many replicates did they use for the validation?

Also, I saw mentioned somewhere that the criteria for PET testing of antacids is different, what are the differences for antacids if any?

And last question, for example the pass for yeast and mould is 1 log reduction, what is the guidance for rounding is it 1 or 2 significant figures. For example if I get a result of 0.96 log reduction is that 0.9 or 1 log reduction i.e pass or fail?

A1: Hello Isabel - the rounding is done per the specific pharmacopoeia. In Europe it is different than in the US - so it depends on where you are registering. But each of the guidelines should have a section on rounding.

For registration, we did validations with three different lots of the same product and three lots of the same media. For intial testing, you can test just one lot, but for registration triplicates are required.

Otherwise your method sounds pretty good. Usually you don't need to go beyond the 1:10 dilution, but it depends on your product.

A2: I have also questions regarding PET. Can we apply PET testing on any product like capsules or tablet. like triphala or boswellia capsules. what will be the method then for solid samples, shall I dilute them in saline or what approach should I take up make this test successful.

A3: I'd not contrive an application of PET that has no relevance to the product or its application. Why do you feel compelled to test these products?

A4: Yes, we are having a few problems performing PET on this product alright but the request to perform PET was from an external site so unfortunately we are left with trying to figure out how to do it.

The pass fail criteria on another method I saw for validation was a factor of 2 but I don't know how this criteria was decided on. The criteria in the European Pharma as far as I am aware is a factor of 5 but I imagined that this is a very wide range?

A5: You validate your PET for each specific product and preservative and test organism by validating the neutralizing diluent media you are using. I don't have access to EP righ now, but in the USP there is a procedure described on how to do it, and what the acceptance criteria are. You have to be able to recover a certain percentage of the inoculated test organism in the presence of the product in the neutralizing media.

If you are talking about acceptance criteria for the PET itself, there are various pass/fail criteria specified in the EP for each product.

A6: Sorry, but you need to read what is stated in the Ph Eur text.

Who was the external site that asked for the study to be done? If you undertake this type of study you will generate some data that will not be helpful and which will then be very difficult to explain away.

A7: You would be surprised at how many ways organizations contrive ways to do PET tests and on the myriad different products that do not need that specific testing. Some are simply looking to see if there is any anti-microbial activity in the product and others have been told by outside groups (regulators or their customers) to do the test. Rather than using an appropriate method (like ASTM or AOAC), they use what they are familiar with - the compendial PET tests. In many of these cases we expend a lot of effort trying to convince them to test using alternative methodology, but often someone, somewhere, often higher up, has made the decision for them. We try to educate as much as possible but often we end up performing the testing. As for a pass/fail criteria, we do not report any (since there are none) and issue the report with a statement explaining that.

A8: Guess I'm not surprised. Certainly understand that you can't determine pass or fail - can imagine that data from contrived ill-conceived tests often leaves 'em wishing they'd never demanded the testing.

CMO Contamination Issues

An interesting Class I recall is listed in the current FDA Recall Report which highlights the difficulty in working with CMOs if you do not pay sufficient attention to their quality and contamination control systems.

The company (Company 1) outsourced the manufacturing, and then the CMO had sterility issues with a different client (Company 2) for which it manufactured the same products. This difficulty then splashed on Company 1's products, and Company 1 is out of luck.

The press release for the recall is at http://www.fda.gov/Safety/Recalls/ucm214850.htm - this is just another example of the critical need to qualify, regularly audit and closely oversee your contract assistance.

A1: The CMO involved had additional recalls beginning back in July. The contamination was identified primarily as mold but the interesting part to me is that to the best of my recollection, the products involved are terminally sterilized. I would suspect that the lots involved in the recalls are just the tip of the iceburg! There are good CMOs out there (I worked for one in another life)and Scott is absolutely correct regarding the critical need to qualify, regularly audit and closely oversee your contract assistance. This holds true for both contract manufacturing and contract testing.

A2: The way to avoid this risk is by conducting effecient audit on CMO, including the following:
1. Validated cleaning procedures
2. Change Control Process: For example, specific processes may require modifications in the standard operating procedures (SOPs) (e.g., modifications of environmental monitoring).
3. Engineering Department
4. Management Strategy: seniority of the personnel involved in the project and a low turnover rate of key personnel.

TOC cleaning validation

I need to perform a verification of a cleaning process of an incubator via TOC procedure. This procedure will have a triple rinse after the cleaning agent is used (sterile water for irrigation will be used). Could you please give me some insight as to acceptable criteria to meet for this test?

Validation of Isolators

We are going to take an isolator into use and I should appreciate if somebody that has experience of isolators could tell me how to perform the validation of the products.
Is it enough to test possible penetration of the H2O2 into different package combinations (vials /rubber stoppers/ampoules) or should all products be tested separately?

A1: Are you using the isolator for manufacturing/filling or for sterility testing? If the former, studies should be conducted to determine the worst case situation for residual VPHP to enter product containers and testing on the filled product should be undertaken. This can include analytical testing of the filled product to ensure that changes to the formulation have not occurred. If you are talking about sterility testing, you can determine the ability of VPHP to enter the product packaging by a number of methods including absorption studies using a suitable diluent (in the package) and testing the diluent for the presence of peroxide. Each type of container closure system should be assessed. If peroxide gains access to a particular container closure configuration, then the impact to each product on the outcome of the test may need to be established.

A2: If you are going to use it for sterility test, you need to show that the H2O2 does not penetrate your product and media/diluents/rinses. Also you need to do penetration study and residual test. Also do all possible configurations so that you cover all the products tested in isolator. you need to establish the cycle time for the exposure and then ventilation to remove hydrogen peroxide. It is as cumbersome as autoclave validation.

A3: Each product package type, which includes your supplies, types of closures(septum closures, screw capped bottles etc.), if you use any media plates verify the packaging, H2O2 will penetrate through paper

and even some plastic packaging, foil is little resistant. It depends on your concentration and total exposure time.

Now there are commercially available media plates, even liquid media which comes in foil packages or specifically made to use inside isolator. Manufacturer's might be willing to provide you data on how they performed their tests.

You should also consider what if your initial cycles fails for some reason, would you replace all the material inside with fresh material or would you leave them inside and start your H_2O_2 cycle again. Keep in mind the exposure time would be different, you are basically exposing them to two cycles.

It is extensive validation and you should place only the validated packages/material inside the isolator to use with your studies.

Audit report

Do the CGMP regulations permit the destruction of an internal Quality Control audit report once the corrective action has been taken.

A1: You are not supposed to destroy till the stipulated period set by your company for preservation of such documents is completed.

You can do so after the completion period , in India it is 5 years for Drug department and product related documents .

Even then such documents shall be usually preserved as a reference for future .

Stamped " For Reference for Future "

A2: Please be informed that,
Many of the regulatory audit authorities ask for the internal audit or will ask for self inspection procedure and its corrective actions taken and implemented.
You can keep these compliance report (Completed) with you only for regulatory audit purpose.
Showing all your internal audit report builds a good and transparent image of your organization.

Gel Clot Alert/Action Levels for WFI

Could only from those who use gel-clot for endotoxin monitoring of WFI reply? Do you have an alert/action level for e-tox? Even if "no", please reply. If yes, what are your levels based upon?

A1: With WFI and a compendial limit of <0.25 EU/mL, it's normal practice that ANY result which gives a positive result at the LAL reagent sensitivity-- normally 0.03-0.06 EU-- is worth noticing (i.e., "alert" level); WFI shouldn't produce any reaction whatsoever. As for Action Limits, usually the compendial limit is the Action Limit and Specification-

Where Alert Limits can be really invaluable is in monitoring of the feed-water to the WFI system- DI water or purified or whatever feeds the still. Problems and endotoxins are far more likely to develop in these systems than in a properly-designed and maintained WFI system, and monitoring the feedwater with established Alert Limits can go a long way towards catching problems early before they get serious.

A2: We do have an alert limit in 0.125 (as it is the sensibility of the LAL reagent). The action limit although is 0.250. When a positive is found in a sample, we make a two fold dilution to demonstrate or not the 0.250 limit.

A3: you can fix half the value as alert limit

A4: Our action limit is 0.25 EU/mL (compendial) and our alert limit is 0.5 EU/mL which is the reagent sensitivity. The limits are in place for PW and WFI.

A5: e-tox limit is fixed for wfi(0.25 EU/ml).we can't measure endotoxin limit from gel clot method.

A6: You can calculate the Endotoxin concentration from gel clot method as per the following formula:

Endotoxin concentration= Dilution factor of end point dilution X Sensitivity used

For example:
If your water give +ve result at 1:4 dilution when using 0.03 EU/ml sensitivity; the Endotoxin concentration will be as below:

E.C = 4 x 0.03= 0.12 EU/ml which is less than 0.25 EU/ml

Regarding Alert limits, you can establish your alert limits based on the Endotoxin limit of your products while for action limit it will be > 0.25 EU/ml.

A7: Based on the Validation data and previous history of WFI System Monitoring for Endotoxin Content, For Gel-clot LAL we can assign the Action Limit as 1/2 of the Official/Pharmacopeial i.e. 0.125 EU/mL before it become problematic and difficult to control. And also a Alert limit as 1/4 of Official/Pharmacopeial E.L. i.e. 0.0625 EU/mL to keep an eye on the trend from where it starts going up.

Note : Official/Pharmacopeial - USP E.L. for WFI is 0.25 EU/mL

biofilm in water

What do you know about the formation of biofilm in the water? How to remove it from the system for purified water.

A1: I'm just doing a university study on biofilms in water systems. For cleaning, disinfection using chlorine or hot flushes may help to reduce alongside UV irradiation of the water. However, once formed it is difficult to remove without replacing the system. Best way to prevent it from getting into your purified water system I would suggest filtering the water before it enters the system.

A2: Having just had to deal with this issue I can tell you that you will probably not be able to rid your system of a biofilm you will only be able to manage it. I found a lot of good information at this site:
http://www.biofilm.montana.edu/
It is the university's center for biofilm engineering.

A3: As [name redacted] said, is very difficult to remove biofilm once is developed in the water system, although prevention is the better way. My experience with biofilm removal, has show a success when I perform a biofilm removal with Peracetic Acid 200ppm. First step is to clean the system with alkaline detergent, rinse through and after very good drainage, saturate the pipe lines with peracetic solution 200 ppm, actuate the valves, close them and let the acid peracetic in the system for a minimum of 8 hours. After this time, the PA can melt the LPS layer of biofilm and is crucial to perform a new C&S.

A4: We have already tried with chlorine, and hot sanitation. Finally we were doing treatment with ozone, but we still have increase of microorganisms (most P. aeruginosa) in sample of purified water. My team and me do not know what else to do.

A5: thanks a lot everyone for your advice and suggestions. Do you have information that ozone should kill "100%" P.aeruginosa in the water? I found this information. Because I do not understand how P. aer. still

exist in the water system after a 24-48 hour treatment with ozone??? Maybe I will try that process with peratcetic acid 200ppm..

A6: It can be very difficult to remove depending on how many layers of lipopolysaccarides have formed on the inside of the piping. We have utilized Sodium Hydroxide followed by thorough rinsing, followed by Peracetic Acid followed by rinsing again until the TOC levels are back to normal. If the layers of LPS are many then what happens is a few layers slough off and fresh microorganisms are free to colonize the system again. If the layers are heavy you may need to replace piping in particularly bad areas. Boroscoping can be used to confirm if the you are successful in Biofilm removal before having to resort to piping replacement.

A7: I agree with the responses you've gotten from Elaine, Jan, and Sandra. I've got a couple of additional comments for you:

To [name redacted] point, chlorine is part of the solution, but in itself, will not control or prevent biofilm.

To [name redacted] point, the Center for Biofilm Engineering has a wealth of information on biofilm problems in water systems. I'd recommend contacting Dr. Paul Sturman there and he can point you in the right direction as far as contacts within the Center.

To [name redacted] point, peracetic acid is a good choice, and she's right that you'll need an alkaline cleaning agent as well. I'm sure TC Soli will add some comments to this once he gets on line.

A few additional comments:

First, check your system engineering. The system needs to be constantly moving at at least 5 ft/sec (1.5 m/s) linear velocity. I'd also recommend that if you can bump up the flow rate before any chemical cleaning, you may physically tear away any biofilm that's become accustomed to the standard system flow rate. Once you tear some of the loose stuff off with an unusually high flow rate, you'll have a thinner biofilm that allows your chemical cleaning to be more effective. Even more effective (although not necessarily practical) would be to run the system in reverse flow to help peel away any loose layers of biofilm. By the way, no matter how high your flow rates, you'll always have a boundary layer of no flow along the pipe wall that is thick enough to support biofilm growth. Now that biofilm has established itself, you're going to have to actively manage it from now on. However, the higher the flow, the thinner the biofilm.

Next, check your valves, pumps and other fittings and components. There's no such thing as a sanitary ball valve, and you'll need to look on the backside of valves and pump seals to see if any water has leaked into the wrong places. If water gets into the wrong side of a valve or seal, bacteria can get back into the water system. It goes without saying that deadlegs should be removed. Also, I've found enhanced biofilm growth when the water temperature gets above ambient (which may happen due to seasonality or friction due to water flow).

The exopolymer that biofilms produce varies in chemistry based on the system design, the kind of nutrients that are in the micro-environment, etc. The exopolymer can be protein, glycoprotein, polysaccharide, lipid, ester, etc. This is why bleach alone doesn't control biofilms, and in fact won't completely penetrate a biofilm. Unless you know the chemistry of your particular exopolymer, it is best to cover as many bases as you can by using various cleaning agents. Further, these should be surface cleaners to help dissolve through the exopolymer. Once the surface cleaners are done, then follow up with sanitizers, which aren't designed to remove organisms but should kill any organisms that remain after surface cleaning.

I'd also suggest running your chemicals through at a low flow rate, not letting the chemicals soak stagnant in the system. Keeping the chemicals flowing will help remove consumed chemicals and hydrolyzed biofilm from the pipe surface, and continually expose lower levels of biofilm to fresh cleaning chemical.

A8: First you need to make sure you are dealing with a system issue and not a valve, hose, or sampling issue. Remediation efforts would certainly be different depending on the source and extent of

contamination. How often do you sanitize the water system? What organisms and numbers are you detecting? Is there a trend? If so, when did it start? What is the flow velocity (Reynolds number)? Do you have any dead legs that could be "feeding" biofilm cells into the system? This can actually happen during a system modification or addition. Make sure you ask someone to walk the system for any changes. Have you sampled the RO water?

Extended (2-4 hrs) heat sanitization (T > 85C) would certainly take care of the biofilm cells, however, it would not remove biofilm mass attached. If your flow velocity is very high, like most water systems, it is unlikely that a significant biofilm mass could form in the minute laminar layer present in the pipes.

Before using harsh chemicals to take care of the problem, you need to understand what is going on with your system and investigate potential sources of the contamination.

A9: You are right. Just like the old saying that an "ounce of prevention is worth a pound of cure". A purified water system must be maintained and monitored constantly.

A10: For sanitization of PW system you can use chemical methods-oxidizing agents such as halogenated compounds, hydrogen peroxide, ozone, or peracetic acid.Halogenated compounds are effective sanitizers but are difficult to flush from the system and tend to leave biofilms intact.Compounds such as hydrogen peroxide, ozone, and peracetic acid oxidize bacteria and biofilms by forming reactive peroxides and free radicals (notably hydroxyl radicals).The short half-life of these compounds, particularly ozone, may require that it be added continuously during the sanitization process.Hydrogen peroxide and ozone rapidly degrade to water and oxygen;peracetic acid degrades to acetic acid in the presence of UV light.
Or
Thermal approaches to system sanitization include periodic or continuously circulating hot water and the use of steam.These techniques are limited to systems that are compatible with the higher temperatures needed to achieve sanitization, such as stainless steel and some polymer formulations.Although thermal methods control biofilm development, they are not effective in removing established biofilms.

A11: Have you confirmed that it is the water system per se or in the sample valve?

A12: Sorry for the late response. You've had lots of good advice thrown at you, but we don't know what your system looks like or its compatibilities with sanitizing agents. However, a general rule is that if you get grow-back within a few days or a week after a chemical treatment, you probably killed only the surface layers of the biofilm and the survivors underneath rapidly re-grew from the nutrients provided by the overlying dead biofilm cells! It's so obvious, but no sanitant can kill what it does not contact. And if it is not completely killed, it will be back in short order.

So the main thing that prevents complete kill is lack of penetration, perhaps due to insufficient concentration, insufficient contact time, insufficient oxidation potential or aggressively, or insufficient access. Though all of these are likely in play, my guess is that the problem is made much worse by insufficient access. Biofilm that that has had a long time to develop will be growing in ALL the hard to reach cracks and crevices at every gasket, joint, rough weld, valve, stress-crazed pipe surface, and low flow area throughout your whole system. An often overlooked area is the moist headspace dome of the storage tank in a recirculation loop that doesn't have spray balls on the return at the top of that tank to distribute sanitant across those tank surfaces.

It's hard to say where your Achilles Heel happens to be with no system description. Nevertheless, I usually recommend using a circulating hot water sanitization (at ? 65°C at all points) with flushing of that hot water through every sample port, use point, side leg, control valve, bypass piping, or whatever in your whole system and maintain that temperature or higher for several hours in order to heat up all those nooks and crannies and kill the cells in the biofilms in those crevices that are otherwise hard if not impossible to reach with chemicals.

However, just the hot water is not going to remove the dead biomass that will serve as food for rapid regrowth from fresh contaminants. Removing that dead biofilm mass will require strong oxidizing and/or hydrolyzing cleaners with more flushing with essentially sterile water to keep from re-inoculating the

system. You might want to try a mixture of caustic and hydrogen peroxide (at least 1% each). It does a pretty good double whammy on biofilm with 2 modes of action at once. Dead and gone is the objective.

Once it is gone, then you can maintain that "clean" state by feeding that system with essentially sterile water by any number of ways. Many people ozonate their tanks and then kill the ozone as it leaves the tank into the distribution system. Others use UV followed by a 0.2 or 0.1 micron-rated filter and change the filter periodically (how often depends on the nature and level of bio-load passing through and hopefully being killed by the UV with only dead bodies being trapped by the filter. DO NOT just use a filter since bacteria will get through a 0.2 filter in probably a week or less. Putting a UV ahead of this filter will allow the filter to last many months before bacteria can get through it.

If your system cannot tolerate the suggested hot water sanitization temperatures and you have to rely on chemical sanitization, then crevices where biofilm can hide from the sanitant (even in the best constructed and designed system) will be your nemesis, and persistence will be your only recourse to try to keep it knocked down. You will probably never completely get rid of all the biofilm, but you may get rid of you Pseudomonas aeruginosa problems.

A13: To eliminate the sampling environment you can purchase a sampling device from Millipore called a micropressure, this will allow the water to filter through the device and not be exposed to the atmosphere you then place the filter on to the agar plate in the lab.
To eliminate the sample valve you need to open it and inspect it for contamination.
If you can show that the high counts you are getting are from the loop and not the valve or the atmosphere then you need to consider using hot nitric acid to remove the biofilm.

A14: The Ozone per se must eliminate P. aeruginosa, although, depending the thickness of biofilm layer, the ozone may not penetrate, and you will have P. aeruginosa counting!

First of all, the word is to eliminated the Biofilm and after that, kill the bugs! Peracetic will help you a lot in this process.

A15: Hi, im also interested in the biofilms since we are having problems with our water system. We're currently using a RO water system wherein the permeate is still ozonated as it goes to our product. Our current cleaning regimen of the membranes are base, acid and lastly peracetic acid as sanitizer. after an hour of chemical cleaning, results show that water is negative for any bacteria or fungi, however, after 20 hours of continuous run, we're already getting 200+ cfus in HPC.

i will take note with your inputs as we try again to validate our water system. would it be effective that we pass our ozonated water (0.3 ppm) in UV? or we pasteurized our pdt altogether? your thoughts pls.

A16: Iam agreed with [name redacted]. At first you have to sample the RO water and source point to know from where the contamination arrived.

if your sanitization is once in a week i would suggest to do thrice a week till the problem solves.

During sanitization you have to drain the hot water from sampling ports. continue the sanitisation for 2-5 hours.

One more thing you have to remember is to know the RO membrane condition. if it is having any holes pl. replace.

Check the Flow rate of the system. make the system more turbulent to remove the dead mass.

Doing a harsh chemical treatment is not a good option.

A17: I think before you "eradicate" the biofilm, you first need to understand the root cause for this:

Are there design issues with the storage and distribution systems (ball valves, dead legs, split loop with low flow/velocity)? Ball valves will always have some leakage of water behind the ball, into the seats. This will eventually form a biofilm. Dead leg piping will allow for low velocity of water, which will allow for planktonic cells to become sessile and form biofilm.

Are there any check valves on the storage and distribution systems which would allow for stagnate water which in tail will allow for biofilm formation?

Are operators using hoses to dispense the water for cleaning/sanitization/batching where they leave hoses connected to the use point. One will never be able to allow water to be removed from the gasket interface with the hose and clamp. Even a few drops of water on the gasket, left un-sanitized, will form a biofilm.

Was your system shut down at some point, where pumps and use points were left with stagnate water?

Do you use the water from your storage tank to sanitize the tank and distribution loop? If so, this is suicidal. Sanitization is not sterilization and you will always have injured cells surviving the sanitization process. If you bring that same water used for sanitization back to the storage tank and remove the sanitizer (ozone, heat), those injured cells which survived will revive themselves and feed off of the dead cells and proliferate.

Is your distribution loop continuous flow, or are there areas where there is no flow? Example, having a valve on the top of the loop and a branch of piping leading downstream from the top valve/loop. This design will never allow the leg of piping below that closed valve to be sanitized, unless you open each valve during loop sanitization and run the water through each branch into a tank or waste.

These are just some examples of issues I see. There are more, but not having an idea of your system, I would be impossible for me to diagnose the root cause.

EM limits in Grade B cleanrooms

Working to the limits in the orange guide the Grade B limits for settle plates are stated as 5cfu/4hours. At my current place of work we interpret this as meaning that 5 and above is a breach of the limit and an investigation is raised. We also pro-rata the limits against 4 hours, for example if a plate is exposed for 1 hour the limit is adjusted to ¼ of the maximum 4 hour limit, therefore 5/4 = 1.25cfu and this is rounded down to 1cfu (as you cannot count 0.25cfu).

I have seen these limits interpreted differently at different places (i.e. no pro-rata of the limits and 5 being the maximum allowable cfu and anything over 5 is an action) and was wondering how people interpret these limits in their own cleanrooms and if they have any guidelines which support their views. This practice was recommended by an MHRA inspector in their last visit so it is unlikely we will change, but it would be nice to get a view of how these are interpreted across the industry and any literature that accompanies practices.

Inactive virus removal by HEPAs

Does anyone have any information on the effectiveness of cleanroom HEPA filters at removing inactive viruses? Is there any scientific information available on this topic?

I am looking at a facility handling both biologics and non-biologics, and want to know if a shared air handling unit is appropriate, given suitable HEPA filtration.

A1: HEPA and ULPA filters act more like depth filters than membrane filters - there will most likely be a reduction in viral load, but these are not absolute filters. You will be best served by a once-through HVAC system for the biologics suite.

nonviable monitoring

We are sterile beta lactam manufacturing plant. Should we perform nonviable environmental monitoring during the aseptic powder filling under powder filling machine (class A), despite the fear of clogging the particle counter suction tubes &/or action limit excursion due to dust produced from the powder filled in vials.

Can we give up the particulate monitoring of air during operation under aseptic powder filling, or is there a solution for this problem.

A1: It would be suicidal to use a particle counter while you are running your filling operation. More than the tubes you will fill the optical chamber of your unit with powder.

A common practice is to sample before and after filling. You could install a counter in the background B area near the filling machine.

A2: You should set the particle counter probe up by where the fill is taking place, but slightly higher so that the powder is not directly above the probe. This way you will be able to monitor the actual air above the powder fill and not the air mixed with the powder, which will give you higher particle counts than desired.

Sterility test washing cycle

I am writing to you this time regarding the following text of European Pharmacopoeia (paragraph 2.6.1. Sterility)

"If the product has antimicrobial properties, wash the membrane not less than 3 times by filtering through it each time the volume for the chosen sterile diluent used in the method suitability test. Do not exceed a washing cycle of 5 times 100ml per filter, even if during the method suitability test it has been demonstrated that such a cycle does not full eliminate the antimicrobial activity".

The statement above means that we use a washing cycle of 5x100ml in routine even if we haven't demonstrate microbial recovery during method suitability studies?

If for a microbe e.g. Bacillus subtilis we can't eliminate the antimicrobial activity with a washing cycle of 5x100ml can we assume that the product is not likely to be contaminated with the given species of the microorganism?

A1: That is correct. I asked the question to both USP and EP. We were recommended to use max 5*100 mL rinse cycle.

The response I received from EP to the following questions was in red below:

The questions are:

* Should all exhaustive measures must be attempted, including exceeding a wash cycle of 5 times 100mL to get recovery during the method suitability test, but not to exceed 5 times 100 mL wash during the routine test of the samples? OR

* Even during the method suitability test, do not exceed a washing cycle of five times 100 mL per filter even it doesn't eliminate the microbial activity.

You should not exceed the washing sycle of 5 x 100 ml even if it does not eliminate antimicrobial activity. An excess of filtration can dammage germs, which will no more grow in the prescribed conditions.

A2: I'm not agree
The maximum is 1000 mL per membrane of test and it is written 5 x 200 mL in the EP.

A3: I have two questions:

1. What is the product (is a liquid, is a made of sliver, etc.)?

2. What are you washing with?

Disinfectant

Is it mandatory to use same type & concentration of disinfectant in Microbiology lab what the production people are using in their aseptic processing area.

A1: Necessarily not required.
For the simple reason you may not have the same organisms in Production and in your LAB.

A2: I do agree that microbial species obtained between a laboratory setting and a production area could be different, If you have this concern of the microbial species being different, why not try a different disinfectant that contains a different active ingredient. However, why would you deviate from the use-concentration of a disinfectant that a manufacturer has recommended for use with their product. Besides the active ingredient, there are other ingredients in the disinfectant concentrate that may play an active role in how effective a disinfectant can be on a surface that a manufacturer has optimized by diluting their formulation to a particular use-concentration. If you are going to deviate from the directions in how to prepare the use-concentration recommendation of a disinfectant by a manufacturer, have you qualified these different use-concentrations of a disinfectant by conducting microbial disinfectant efficacy studies with your environmental isolates?

A3: it is not necessarily required, because for the aseptic manufacturing area you have special requirements like sterile disinfectants, rotation and one sporicidal disinfectant (see EU GMP Guideline Annex 1 and FDA Guidance for Industry "Sterile Drug Products Produced by Aseptic Processing"). For your lab rooms, I think it is not necessary to fulfill this requirements, but if you use class A working benches or isolators e.g. for sterility testing, you will have the same requirements.

Inhibitory property of MacConkey broth

According to European Pharmacopoeia paragraph 2.6.13 (current harmonized method) in order to demonstrate the inhibitory property of MacConkey broth you should inoculate the medium with at least 100CFU of Staphylococcus aureus. After the appropriate incubation period no growth of S. aureus should occur.

When we inoculate MacConkey with S. aureus (100-200CFU) we observe that after the incubation time there is growth of S. aureus!

The manufacturer (Oxoid) also states that there is turbid qrowth, but there is not acid and gas production. Should we use another microbe in order to show the inhibitory property of MacConkey e.g Bacillus subtilis?

A1: I would research other vendors.

A2: I have used MacConkey Agar for many years and Staph. aureus should not grow on the media. MacConkey broth I have not used. I would use media from a different vendor and see if the problem persists. Bile salts and the dyes are inhibitory towards Staph. One thing to try is add agar to the broth and see if the media is inhibitory as solid media. I have never seen Staph be able to grow on MacConkey media. If you change the organism you are deviating from the EP.

A3: I agree , I have not seen Staph growing in MacConkey agar. If they observe growth, probably they should check the sterility of the culture they are using, may be it has some contaminant other than Staph. One way to check the sterility is to plate the growth on Mannitol Salts agar and see if they get the characteristic Staph colonies.

A4: Reading this again I had a thought. You should streak out the Positive Staph. aureus growth to verify that it is growth and not turbidity. Also Gram stain the from the media broth and the streak plate (if growth occurs) to verify that you have not contaminated the broth with a Gram(-) organism. The gram stain should indicate Staph aureus(G+ cocci clusters).
I am betting you have precipitate from bile salts or contamination. Do you check the pH of the media? Bile salts precipitate at low pH.

A5: We had a similar problem and it was because we were using the wrong MacConkey agar. There's MacConkey without salts and No.2 and No.3. I can't remember of the top of my head which one is specifically made to inhibit Staph - I think it's No.3, but your Oxoid rep should know.

A6: No, in several years of testing MacConkey Broth, I don't recall ever obtaining Staph. aureus growth whilst performing the inhibitory test.

ENDOTOXIN CALCULATIONS

When we are analysing endotoxin content in a product for which the endotoxin limit is established in the pharmacopoeias in terms of EU per mg of a different chemical formulation, should we correct the results taking into account the molecular weights of both products? As an example, endotoxins content specification for Methylprednisolone sodium succinate for injection is 0.17 EU/mg of Methylprednisolone, the same with Hydrocortisone Sodium Succinate for injection (EU/mg Hycrocortisone).

A1: Generally, it is not necessary to correct results for the molecular weight. I do not know of any product for which the endotoxin limit is expressed per mole (or millimole). However, there are some for which the limit is given per milliEquivalent (mEq), which is similar in principle. In such cases you will need to know the concentration of the product in mEq/mL and can then express the limit in EU/mL for that concentration. As long as the limit is expressed per mEq and the concentration is expressed in mEq (even if that is only a portion of the drug molecule) per mL, there is no need to make any corrections.

Understand that when testing the finished drug product you will be testing it against an endotoxin limit calculated as described in the Bacterial Endotoxins testing chapters in the USP or EP. Although that limit is expressed per unit of active pharmaceutical ingredient (API, or drug substance), you are actually testing the whole finished product, which includes other components of the molecule, excipients and the container-closure system. You do not need to make any corrections for the molecular weight etc. because the finished product will only meets specification if the sum total amount endotoxin contributed by all components is less than the endotoxin limit calculated from the maximum dose of the product. A limit expressed in EU/mL as described above will account for all components.

When setting the endotoxin limit for the API (as opposed to the finished drug product) you will need to bear in mind that endotoxin may be contributed by other product components. If the limit for the API is set at the limit given in the pharmacopeial monograph, there is no allowance for any endotoxin in other product components. Consequently the limit for the API should be set at a lower (more stringent) level to allow for possible contribution of endotoxin from other components. Similarly, do not treat each excipient as if it were a separate product and calculate the endotoxin limit based on the dose per kg of each. This would

allow the individual components to contain an amount of endotoxin below the limit but when combined, the formulated product might exceed the applicable generic endotoxin limit (< 5 EU/kg body weight for parenteral products other than those administered intrathecally and radiopharmaceuticals).

Lactobacillus/Acidophilus recovery on the Soleris Units

We are looking at Lactobacillus/Acidophilus recovery on the Soleris Units (Neogen). We are using a Lactic Medium Vial (ph-6.2-6.5) to add our dilutions to. Is there a prep procedure that works best at recovering this bacteria on these units. Thanks in advance.

This medium does not seem to be consistent. IS there any alternative mediums for testing Acidiophilus on the Soleris units.

Micro testing on finished product

When performing microbiology testing on finished product, does anyone perform reanalysis/re-testing of sample preparation (original sample preparation is retained) in order to assess the accuracy of the original suspect/aberrant result?

A1: How long would the original sample need to be retained in order to retest it? What effect would that have on the bioburden viability or count?

A2: You only retest original sample if the initial test has problems and use retest as a conformatory test as a part of investigation.

A3: But the sample will be considerably older if it is retained for the test/incubation/reading period. This could have a significant effect on the viability of any organisms present so the results of the retest might not be valid, particularly if there is antimicrobial activity in the product. Thus the earlier comment in the earlier posting.

BIOFILM Quantification

A colleage is trying to undertake some work involving biofilms. He wishes to try to assess the efficiency of removing (without killing the organisms) a biofilm from pipework.

The trouble we have come across is how do we quantify the number of organisms attached to the pipework before any mechanical removal of the biofilm has been performed and how can we then quantify the remainder of film on the pipe?

If anyone has any experience in trying to quantify biofilm in situ, we would be grateful for the help!

A1: Biofilm in pipework is a tricky measurement issue. All too frequently, there are few cells and lots of exopolymer in the biofilm, which may give rise to the question of whether the cells or the exopolymer is the more important target. Further, isolating the cells from the piping and their encasing exopolymer and getting them to grow in culture can also be a problem.

I favor use of removable witness plates, followed by analysis by surface analytical chemistry methods, particularly infrared spectroscopy. The biofilm that forms on the removable coupon stays in place on the coupon for analysis - no worries about recovery efficiency of swabs. Peak height in infrared analysis correlates to mass of the biofilm. Looking at peak heights before and after a cleaning treatment is a good measure of how effective the cleaning treatment is - but reflects primarily the impact on the exopolymer, as bacterial cells tend to be too small to generate much of an infrared signature on a pipe surface. Exopolymer, however, can produce excellent IR results.

A2: There are some newer rapid methods that enumerate organisms based on the ATP levels recovered. Bioluminescence is usually involved.
You could calibrate the system with your Biofilm using plates with wells. For a certain area of Biofilm you get this level. The organism has a dimension (length and width) so you could estimate from the average area how many organisms are present.
With bacteria you usually can only estimate, even a standard plate count is an estimate because you are measuring CFUs and not individual cells. I am not sure if you can enumerate cells in a cell sorter and count them.
It would be interesting to see if a surfactant or a chemical like Formalin would break the Biofilm up to individual cells.
If you do find that compound you could make a lot of money.

BET on concentrate dialysate

I'm testing 2 concentrate dialysate, where one of it is acidic and another is basic.

The pH range for BET is within 6 - 8.

I found that to adjust the pH of the acidic product with 1M NaOH to within the range will end up diluting the sample up to 15%! So, my thought is to use 3M NaOH to adjust the pH. Does anyone have better suggestion?

2/ For the basic sample, the more acid i add, the higher the pH i get. How would you all handle this type of dialysate sample?

A1: For acid products I use a more concentrated phosphate buffer pH 7.2 as a diluent.
Previously I determine the concentration of phosphate which the final pH (with the product) is within 6-8, and I use it as a diluent (I don't adjust the pH).
You need to perform the toxicity test for this new diluent, and to validate the method, of course.

Sometimes basic samples don't need neither adjust the pH nor to use a concentrated buffer. You can determine the method after validation.

A1: Because LAL has a natural buffering capacity, we typically find that we do not have to adjust the pH of most samples, including dialysate. The suggested pH range of 6-8 refers to the pH of the reaction mixture, which is sample + LAL, not sample alone. Because LAL is so sensitive, one can typically dilute the sample out to the MVD with LRW which will overcome most interference problems.

Detection Limit of <1cfu/mL for MLT

Has anyone applied a detection limit of <1cfu/mL to USP Microbial Limits Testing and if so, what was the rationale? Would you not want to consider the Level of Detection as demonstrated by growth promotion in which you use 10-100 cfu and therefore have your limit of detection set at <10 cfu/mL?

Rejecting Aseptic Product for EM Excursions

I am interested to find out whether companies who are making aseptic product ever reject batches based on EM data. If so, is there a guideline for how many excursions constitute a rejection? What are the factors that are being considered when making lot disposition decisions?

A1: YES- several places I have been, it was/is entirely possible to reject product batches based solely on aberrant EM results. The term employed is "Lack of sterility assurance", and procedures are written to provide for an overall 'risk assessment' of the entire aseptic processing environment associated with a batch. Things such as: particle count spikes (not necessarily over limits but higher-than-normal or "out-of-

trend"); unusual incidence of personnel gown or glove plates showing growth (again, not necessarily over limits, just atypical numbers of 'hits'); surface samples or air samples showing colonies; etc.; are evaluated as indicating an unacceptable level of risk or exposure of product to possible contamination.

In one company, the initial response to a less-than-encouraging EM picture was to perform a double or triple sterility test (which, of course, gives 2x or 3x the 'assurance' of a single sterility test-- i.e., 2x or 3x nothing). But in other places, batches were placed on hold, investigated, and, depending on the results of the investigation and evaluation, could be rejected, even in the complete absence of a positive sterility test or any other failing batch test results.

A2: In order to know the risk, you could inoculate the product with a suspension of the isolated microorganisms (as a challenge test).-

A3: The point of EM is to determine the state of control of the facility. If you have significant EM excursions (up to QA to define significancy) then the facility has been demonstrated to not be in a state of control. The status of the testing for the finished product is irrelevant - the facility is not in control, therefore the process validation parameters have been exceeded and so the process by definition lacks "sterility assurance". The product is adulterated.

This is one of the reasons that QAU has the responsibility and authority for batch release. It is not up to either the Microbiology or the Manufacturing group to determine if the facility is in a state of control - only the QAU (reference the 21 CFR210 and CFR211 sections for responsibilities). The environmental monitoring data is a major piece of this determination.

A4: Let me offer a view somewhat different from that presented by Scott.

It depends on the purpose of control levels and how they are established.

They should be established such that exceeding them does not require product rejection.

Consider, for example, these quotations from USP <1231> Water for Pharmaceutical Purposes.

"Alert and Action Levels are distinct from process parameters and product specifications in that they are used for monitoring and control rather than accept or reject decisions."

"Action and Alert Levels are established within process and product specifications tolerances and are based on a combination of technical and product-related considerations. Consequently. exceeding an Alert or Action Level does not imply that product quality has been compromised."

"Product-related Alert and Action Levels should represent both product-quality concerns and the ability to effectively manage the...process. These levels are typically based on a review of process data and an assessment of product sensitivity..."

"The levels should be set such that, when exceeded, product quality is not compromised."

These quotations are taken from a presentation I give on trending in pharmaceuticals, includng for EM.

A5: Great to hear from you. I fear that we find ourselves in agreement again.

The PDA EM technical report provides the guideline that the alert level should be exceeded 5% of the time, the action level 1% of the time. Using this as a guide, some level of excursions are to be expected.

I apologize for glossing over this point, but when I said "If you have significant EM excursions (up to QA to define significancy) then the facility has been demonstrated to not be in a state of control." I was not speaking of the expected excursions, but evidence that something has gone awry. In this situation of excess excursions there is "lack of sterility assurance."

The relevant regulatory documents are clear in the directive that the purpose of EM is to gauge the state of control of the facility, the trick comes in determining when that control is compromised. We do expect a well-run, in-control facility to have regular excursions (just not too many!).

A6: I have been involved with two instances where EM data played a significant role in rejecting an asepticly processed batch.

In the first we recovered a cluster of contamination, one sample significantly over the Action Level (20X or more as I recall) and 2-3 others in the same area with passing counts. All samples recovered a common organism, a known pathogen, and one site was considered critical with potential indirect product contact.

The second involved non-viable particle counts that exceeded the action level at one site in a fill room. The investigation identified a bad seal on a moving part of the fill assembly.

A7: What would happen if I retest the product and the result had pass the sterility test but the EM doesn`t? should I reject the batch?

A8: I do not have enough information to answer your question - without knowing your process, your product, and your EM trends the unknown EM readings of a single day are not enough to go on.

If I was in your position, however, I would approach it from two perspectives - that the lab results are valid unless proven otherwise, and that the EM data is there to assist in determining the state of control of the facility.

A failed sterility test is easy - don't release the product unless you can conclusively prove the test invalid. No retest may to overcome a valid, initial failure.

If the sterility test passes, but there are issues with EM, it is not as easy. As has been discussed in this thread, excursions in EM are expected. PDA's "Tech Report #13: Fundamentals of a Microbiological Environmental Monitoring Program" recommends that the Alert Levels for different areas be set at the 95th percentile (or that the planned excursion rate should be 5% in each area for Alert) and that the Action Levels be set at the 99th percentile (planned excursion rate of 1%). Therefore, if the Alert and Action Levels are set correctly, excursions are expected and certainly are not in and of themselves evidence of a problem. However, adverse trends (too high an excursion level, excursions in sensitive areas, too frequent an excursion in a restricted area (for instance if all excursions in a room happen in one spot, the frequency of the excursion is less important than the trend)) should alert a responsible QAU that the facility is not in a state of control, that the facility is operating outside of its qualification parameters and products produced in that facility "Lack of Sterility Assurance". We have ample evidence from recent Warning Letters and 483 observations that this is the position FDA will take (as an aside - PDA/DHI has recently published a pair of books that are useful in reviewing recent enforcement activities entitled "Recent Warning Letters Review for Preparation of an Aseptic Processing Inspection" by Jeanne Moldenhauer that might be useful).

Again, there are two separate measures here - The sterility test as a finished product requirement, and EM as one of the measures of the state of control of the manufacturing facility. A failed, valid sterility test is a clear decision - no retest, no marketable product. Issues in the EM data require thought and analysis with a qualified SME (subject matter expert) helping the Quality Assurance Unit (QAU) in their decision about batch disposition.

There are many experienced people on the list and I look forward to the continuing discussion on this topic.

A9: The results obtained on a sterility test are just one piece of the puzzle used to assess the sterility of the product. Another of the pieces is the environmental monitoring results showing that the facility is in control. So to answer the question if I had failing EM results with a passing sterility test, I would perform an

investigation to show how all the pieces of the puzzle fit together and on that basis make a disposition on the product.

Possibility on Put Autoclave on Micro Work Area (besides Biosafety Cabinet)

My company has bought new autoclave for main autoclave back up. For safety issue, we prefer not to put the new one on preparation area which has been very crowded. My question is: Is there any possibility to put the autoclave on micro testing area, beside biosafety cabinet and what additional action must be taken in order to do that.

A1: I see no reason not to do this. After all double-door autoclaves are used in aseptic filling areas.

What you must do is make provision to exhaust the heat and steam when the door is opened.

Shortening Incubation period on TAMC for Non-Sterile Pharmaceutical Products

Micro lab of my company has conducted validation on shortening incubation period for TAMC (5 days to 3 days then to 2 days) on non-sterile analgesic cream. The validation principle is to examine that the product doesn't inhibit growth of 5 tested microbes (we use microbes same as for TSA Growth Promotion Test) on 2 days incubation on 33 C compared to 3 and 5 days incubation. My questions are: 1. Is the shortening incubation period of micro testing has any possibility to be implemented?
2. is there any pharmaceutical companies have implemented the shortening incubation period and/or rapid micro testing?

Endotoxins and interaction

I have several questions :

- First, i need to spike a product with a concentrate endotoxins solution. I want to use RSE (10.000 IU in the vial). Due to the fact that we should use 5 mL to rehydrate the lysate to have 2000 IU/mL, it is possible the rehydrate with 1 or 2 mL and not 5 mL ? Without problem of stability after because we don't follow the operating procedure ?

- we have a biological product with about 1200 µg/mL of total proteins and with 500 µg/mL of Triton (detergent)
When we spike a big amount of RSE (500 IU/mL) in this product and let it at +5°C during 1 day we have about 80% of recovery
But when we let the product spiked during 8 days at the same temperature we have only 3% of recovery of the spike !!

So thanks for your experiences and proposition for the preparation of the samples to have more 70 to 80 % of recovery and not only 3% after 8 days of contact.

A1: In your first question, I think you may be referring to RSE as LAL(?). If you are using RSE, you need to follow the instructions in the package insert. Here at Associates of Cape Cod, we do manufacture a CSE at 125 ug/vial which can be reconstituted in different volumes to achieve the concentration that you desire.

In your second question, there may be several factors occurring. Are you trying to do a stability study on your product? It could be that your product is binding the endotoxin. Sometimes we see that with proteins and have to use heat treatment. Have you verified that your containers are not adsorbing the endotoxin? You can check that by spiking LRW for the same amount of time that you are storing your samples. Be sure to include PPCs so that you verify that you are not just measuring inhibition.

minimum number of thermocouple /data logger will be used for sterilizer validation

How many thermocouple /data logger will be used for Autocleve and Dry heat sterilizer validation purpose? Any recommendation/Guideline?

A1: The number depends on the size of the chamber. You will need one at the exhaust port, one with product RTD and rest based how you set up your load (minimum 5 per shelf, four corners and one in center). Most of the autoclaves use exhaust port RTD to control the autoclave.

A2: I do not have the TIR in front of me but if you look at it, AAMI TIR #13, one should use 10 sensors per 100 cubic foot chamber volume for loaded chambers and 5 sensors per 100 cubic foot volume in empty chambers.

USP <1111> Interpretation Survey

Please submit to the forum:

USP 32 <1111> Table 1 recommends enumeration limits in exponential terms. Prior to Table 1, the exponential terms are specifically defined as:

10^1 cfu: Maximum acceptable count = 20

10^2 cfu: Maximum acceptable count = 200

10^3 cfu: Maximum acceptable count = 2000; and so forth

How do you interpret these requirements?

A. Our release specification is 20, 200, 2000, as appropriate.

B. Our release specification is 10^1, 10^2, 10^3, and allow release up to 20, 200, 2000.

C. Our release specification is 10, 100, 1000, but we allow release up to 20, 200, 2000 with justification.

D. We have an Alert/Action limit at 10, 100, 1000 requiring an investigation, but may allow release up to 20, 200, 2000.

E. Other? Please define/describe.

A1: The most apropiated is c. but after investigation, justification and corrective and /or preventive actions.

A2: I have checked with USP regarding the interpretation.

'A' is definitely 'No'.
'B' is how it is to be interpreted.
'C' is better for explaining it to non-microbiologists within and outside your company.
'D' - The results are not considered OOS, so an investigation is not required in my opinion.

A3: From my experience with working with people outside the company, option 'C' is the best. We have implemented an Out-of-Trend (OOT) approach to address any result within 50 - 200% of the specification (using the factor of 2 justification in relation to reliability of results). If you establish SOP's appropriately, you can maintain the OOT process separately from the MDD (OOS) process and justify release or further investigation at the discretion of microbiology supervision/management. Another benefit to this approach is

that you can adequately detect atypical results based on history if you maintain a record of your OOT results. For example, if a material/product is testing without an OOT result on a regular basis and then all of a sudden you obtain an OOT result, it is to your benefit to look into that further and decide whether or not the circumstances warrant an MDD investigation. That's my opinion. I'm curious how others in the industry handle the challenges of this USP interpretation when it comes to both people within and outside of your organization.

A4: I will apply the option C

A5: I will vote for 'B'.

Reason:

10 = 1 x 10^1 ; 100 = 1 x 10^2 ; 1000 = 1 x 10^3 and these are not the expressions in USP <1111>.

I'll put in the specification as 10^1, 10^2 and 10^3 and then explain in the SOP to interpret the result with the imprecision factor as 2.

A6: Option C

A7: I will vote for BBB

A8: Option C would be my pick.

validation of camera to take pictures of gram stains, etc

Looking for information about how to qualify a camera to be used for taking pictures of gram stains, wet mounts, etc. I am thinking that we just print the picture as it is taken and not rely on going back to the software to regenerate any pictures. Given this plan, what have others done to qualify their cameras.

A1: For taking pictures of Gram-stains of unknown bacterial isolates, I would take pictures of the controls (e.g. Escherichia coli and Staphylococcus aureus) each time that the Gram-stain was performed to verify that the staining procedure was performed correctly if you are taking pictures of each and every gram-stain of recovered bacterial isolates. Your question concerning the qualification of pictures concerning wet mounts is interesting.   If you are using a wet mount to determine whether a bacterial isolate is motile, I would take pictures of control isolates that are motile and non-motile by taking a picture of isolates with and without flagella every time that this procedure is used. If you are performing wet mounts of mold isolates to conduct mold identifications, it gets a little sticky.   I do not know anyone taking pictures of control isolates to verify the identification of recovered mold isolates. Unless you have an experienced mycologist at your company to perform these mold identifications, it is my opinion that the conductance of mold identifications by using wet mounts and taking pictures of reproductive structures, mycelium and reverse color of mold colonies is always going to be somewhat subjective in backing up your identification call.   With the technology of today, I suggest people use genotypic identification instead of looking thru a microscope for determining the identification of mold isolates.

A2: Good question as we have a digital imaging system as well. Instead of dealing with part 11 compliance issues, we use the imaging system for our microscope as supplemental data for the actual raw data which is recorded in our notebooks. This is mainly for our mycological identification but we have done it for Gram stains also in the past. Our raw data is our technicians observations on the microscope which are written down in the notebook. That is the information that is written in the report, submitted and reviewed by QA. If there is a question about the ID process, the technician would take a digital image of the questionable result and use that for discussions with a supervisor. The slide is not disposed of and is used if further review is necessary. An additional possibility is that we could immediately print the picture as we are observing it. We would print the image on paper with a glue backing and paste that into the raw data notebook, and have the technician initial and date the image.

Seek for Lab to perform Biological Reactivity Test <87>

We are using rubber stopper in our first developing injectable ANDA product and established the specification for the rubber stopper following USP <381> ELASTOMERIC CLOSURES FOR INJECTIONS. We don't have the facility to perform the Biological Tests, which are based on Biological Reactivity Tests listed in USP <87> and <88>, respectively.

Could anyone recommend a contract lab which can perform these tests?

A1: There are several, but I will only give one (biased) recommendation:

WuXi AppTec
2540 Executive Drive
St. Paul, MN 55120
888-794-0077

More information on the website: www.wuxiapptec.com.

A2: Toxicon will be the good option for these kind of study.

A3: NAMSA also offers this type of testing. Mike Hendershot can assist with pricing, lead time or any other questions you may have regarding NAMSA's capabilities. Mike can be reached at 419.662.4427 or [log in to unmask]

Autoclavable Neutraliser for Peroxide / Peracetic Acid Streilant Carry Over

I was wondering if anyone could provide a good reference for an article on neutralising Peroxide and Peracetic acid or has experience with neutralising sterilant (H202 and / or Peracetic) carry over on BI carriers - in this case aluminium strips.

I am aware of the recent posts on peroxide neutralisation by catalase and have used this in the past but given the logistics of the current project situation enzyme deactivation is not a feasible option as an extremely large numbers of BI strips have to harvested relatively quickly.

I'm hoping to use a single neutraliser step or a neutraliser that can be combined with the recovery broth of choice, in this case Dextrsoe Tryptone Broth.

Ideally, the neutraliser solution will be autoclavable and even better if it has a good track record for incorporation into recovery media. Obviously, I will need to validate its effectiveness but I'm looking for something that is equally effective against Peroxide and Peracetic Acid.

At the moment I'm aware that Sodium Thio & DE Broth have been mentioned, is one favored over the other, what has been peoples experience. Any info would be gratefully received.

A1: Is the neutraliser to be added into the TSB for IBs?

If it is so, you can use hermetic containers for TSB. You can validate the seal through a GPT after a sterilization cycle in the isolator.

A2: A very effective neutralizer for peracetic acid (PAA) is sodium thiosulfate...

Unfortunately, this is not very effective as peroxide neutralizer. So I used to prepare catalase each morning and add this to sodium thiosulfate solutions. The sodium thiosulfate solutions are shelf-life stable for months, but you will need to use the catalase solutions within 24 hrs of re-hydration.

Not the most optimal solution but it is effective for neutralization of PAA/H2O2 on BI strips.

Handswabbing procedures for OOT results

During routine (monthly) monitoring of personnel hygiene, one employees' hand swab had excessive TVC counts (>300cfu/swab). At the time the employee was working with product(we have a non-sterile manufacturing facility - the product in question undergoes microfiltration at a later stage)and was wearing gloves at the time. No other employee had similar counts (on the day in question).

Our current procedure states that TVC counts exceeding the action limit should be linked to a non-conformance. In this instance, seeing as the purpose of the hand swabs is to monitor the hygiene of personnel, the counts can be rationally explained(the employee was working with essentially "raw" product) and the risk of cross contamination is now reversed- the product poses a risk to the employee. After an interdepartmental debate as to whether a non-conformance should be raised or not and after the description of the problem, my query is as follows:

IS the best time to perform hand swabs during production seeing as the objective is to monitor personnel hygiene? Considering that they work in a non-sterile manufacturing facility? What is the correct hand washing/swabbing procedure to use when dealing with personnel who use gloves to perform their tasks?

Are there any guidelines/specifications to deal specifically with cases such as theses? Current practice?

Contract Lab Inquiry

Can anyone recommend a contract lab in the Doral/Miami, Florida area that would perform on-site sampling of water for microbiological analysis and environmental monitoring for microbiological analysis?

ETO sterility test

This is question regarding collecting the samples and Biological indicators from ETO sterilizer for Sterility Test. As per BI manufactures instructions, two methods given for pulling the samples or BI from ETO sterilizer. one is after immediate sterilization cycle and another one is after completion of external aeration process. Which one is the best method and how? Please clarify the question and how can we validate the sterility test if the samples collected after immediate sterilization cycle?

how much quantity of sample for sterility testing

Please tell me how much quantity of sample is required for sterility testing (direct inoculation) of sterile Bulk dry powder if I receive one or two containers of 10kgs of BULK API which is to be used in formulation. please give any reference guidelines.

A1: Not possible to apply the pharmacopoeial sterility test with only two containers?

A2: if you have access to USP, go through MicrobiologicalTests<71> for Sterility.Refer to USP32 P.80

A3: Your sample is a bulk powder. That's a raw material. You probably want to perform a bioburden test, not a sterility test.

A4: Refer to USP <71> Sterility Tests - Tables 2 'Minimum quantity to be used for each medium' and Table 3 for the 'Minimum number of articles to be tested in relation to the number of articles in the batch'.

Table 2

Quantity per container	Min. qty. to be used
Solids	
Greater than 5 g	500 mg

Table 3

Number of items in the batch	Minimum number of articles to be tested
Bulk solid products	
Up to 4 containers	Each container

My interpretation: In your case, 500 mg will go in each of the two media and both containers have to be sampled.

The method has to be validated.

Reducing the number of EM sites

I was wondering if anyone can shed some light on this subject. We've been monitoring our facility for a little over a year, and for 90% of the time, our viable air results are incredibly low (0 CFU/m3). I'd like to reduce the number of VA sites, are there any guidelines to follow, or just kind of "wing it"?

A1: The data appears odd. Are you performing sampling after cleaning and disinfecting activities? If so stop. You should be performing EM activities the day before cleaning the room up.

A2: It is not unusual after starting up a viable monitoring program to review the data and number of locations. With a year of data you have been through the seasonal fluctuations, so that would be a god time to do it. You can reduce the number of VA sites based on the criticality of the locations and the historical data. I would suggest a documented rationale that discusses the criticality and the historical data.

USP Identification

1- As you know in USP 2022 there are all details for identification of all pathogens(if applicable) but not in USP 62 (it says only to identify). could we use USP 2022 as a reference for identification for USP 62 ? If not how do we do the identification for the pathogens (S.a, P.a, E.c, Salmonella)? Is there any othere references? Are the identifications in USP 2022 enough for detecting the pathogen? Should we do API automatically (or we do upon request)? Is there any other references in USP for characteristic colonies description of pathogens (except USP 62 and)?

2-If we have a validated autoclave , do we need to do BI (biological indicator) on a regular basis (example: weekly or monthly) ? if yes what would be the frequency and how we can answer to an auditor asking why you control your validated autoclave? any reference you recommend? what if the autoclave is not validated (the cycles)?

3- If we have OOS result for a pathogen(presence of pathogens) and the result of retesting(requested by client) shows the absence, how we explain this and do we need to complete again the OOS report (a auditor is asking for this)?

4- If the temperature of an equipment (eg.incubator) is not within the range after the first reading and it goes back to normal after 1 hour of observation (second reading) do we need to do an investigation or we can accept the result by adding a note on result sheet(or log book).

5- Could we use USP 62 for detection of P.fluorescence, if yes do we need a deviation report? if not do you recommend any reference?

6- Is there any reference which explains the interval of test for challenge test(2,7, 14, 28 days) I mean these intervals are based on what kind of studies?

Issues with Biolog Media

Out of curiosity, have any of you that have experience with Biolog media had any issues with the sterility of your media? We have had several packs of media exhibit growth without being opened, and my department is curious as to whether this is a common occurrence.

A1: I have never had a problem with the Biolog media that we order.

A2: We observed the contamination only once from the hundreds of plates used in the last 2 years. The media was promptly replaced by Biolog.

A3: My colleague provided me with some feedback on this issue... I knew we had experienced some issues...
We have received 2-3 shipments this year alone with growth on unopened plates. Additionally, we've had a couple of shipments with cracked (unusable) stacks of plates. Most recently, one plate in a stack of 10 Malt Extract Plates, was found labeled as "Rambachrom acinetobacter".
In all cases, we have contacted Biolog and received replacement shipments.

A4: Thank you very much [name redacted] - these issues mirror some of our own!

Sterilized 80% Glycerol - Shelf life

What is the shelf life of 80% Glycerol that has been autoclaved?

A`1: In my academic life (i.e. pre-GMP days!) I would keep and re-use all manner of concentrated carbohydrate solutions, including glycerol, until they ran out. With appropriate aseptic handling I never experienced any contamination problems. Of course, being non-GMP at the time, I never validated the shelf-life .

VRBGA+ Lactose

Any body has an idea about the percentage of lactose added to VRBGA media (according USP) 2021 for deterction of Enterobacteria (any reference?)

A1: My old Bergey's says 10 grams /liter - 1%.

A2: 1% of Both sugars Lactose and Glucose are to be added in VIOLET-RED BILE AGAR WITH GLUCOSE AND LACTOSE as per USP <2021>.

A3: Here you go:

violet-red bile agar with glucose and lactose

Sterility failure

While incubating for Sterility if a sample shows growth in TSB/SCDM after 10 days and no growth in FTM then what should be interpreted? Is it necessary that if TSB/SCDM shows growth then FTM must also show growth? Negative control is negative till date. Please share your experience if any.

A1: It is not expected that you would necessarily get growth in both media. These results indicate a potential sterility failure and must have a thorough investigation performed.

A2: The TSB/SCDM and FTM were developed to promote the growth of different types of organisms (e.g., optimum growth temperature, available oxygen, pH, etc...), so obtaining growth in only one of the two media is not all that unusual. Growth in either media signals a test failure and an investigation (to potentially include a retest) should be performed to determine if the product is non- sterile or if there was some error in the testing itself.

FORMULATION OF MICROBIOLOGICAL ANALYSIS

I've problem with interpretation of microbiological results. We have non-sterility production and haven't our own microbiological laboratory yet. So all microbiological analysis are done by contract laboratory. At last time we received some results where about existence of TAMC and TYMC are mentioned - not detected, whereas in the analysis of balk manufacturer answer are concrete number of CFU or less then 10CfU. I want to know you opinion about this question. I think that such results are doubtful and besides that in nonsterile forms declaration about TAMC and TYMC not detected is incorrect.

A1: You are correct. Not Detected is an incorrect way of reporting the TAMC and TYMC results. There are no indications of the dilution used during testing (1:10, 1:100?). The correct result for no recovery at the dilution tested is <10 cfu or <100 cfu for example. I would recommend that you request the raw data sheets from the laboratory at a minimum. This will allow you to at least (hopefully) see what the dilution may have been and potentially save the results although it will increase your paperwork explaining the results. Another strong recommendation is that you have your audit team (along with a trained microbiologist) visit the facility and review the test methodology, quality system and their microbiology qualifications.

A2: I think you can give your result <10 CFU/ml or gm if you have not dected any colony forming unit.When you detect any number of colony then you should multiply by dilution factor and report it as such, for example, number of colony observed in plate-1 is 2 and on plate-2 you have count of 3 then you add both the count and find the average of two plates, finally you multiply the count by dilution factor and report your result.

Bile-Tolerant Gram Negative Bacteria

When completing Bile-Tolerant Gram Negative Bacteria testing, I referred to the USP table for result interpretation. In the USP General chapter <62> the table states different result interpretation than the table in Dietary Supplements Chapter <2021>. I believe the table in chapter <2021> is an error. Could someone confirm this question?

Survival of organisms on stainless steel surfaces

I was wondering if anyone could point me in the direction of any information regarding survival times of microorganisms on stainless steel surfaces or whether there are any studies which have looked into this?

A1: There is plenty of literature out there as this is critical to the food production industry.

Here is an example that might put you in the right direction:

Survival of food borne pathogens on stainless steel surfaces and cross-contamination to foods

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Abstract The retention of bacteria on food contact surfaces increases the risk of cross-contamination of these microorganisms to food. The risk has been considered to be lowered when the surfaces are dry, partly because bacterial growth and survival would be reduced. However, some non-spore-forming bacteria might be able to withstand dry conditions on surfaces for an extensive period of time. In this study the survival of Salmonella enteritidis, Staphylococcus aureus and Campylobacter jejuni on stainless steel surfaces at different initial levels was determined at room temperature. The transfer rates of these pathogens from kitchen sponges to stainless steel surfaces and from these surfaces to foods were also investigated. Staph. aureus was recovered from the surfaces for at least 4 days when the contamination level was high (105 CFU/cm2) or moderate (103 CFU/cm2). At low levels (10 CFU/cm2), the surviving numbers decreased below the detection limit (4 CFU/100 cm2) within 2 days. S. enteritidis was recovered from surfaces for at least 4 days at high contamination levels, but at moderate level, the numbers decreased to the detection limit within 24 h and at low level within 1 h. C. jejuni was the most susceptible to slow-air-drying on surfaces; at high contamination levels, the numbers decreased below the detection limit within 4 h. The test microorganisms were readily transmitted from the wet sponges to the stainless steel surfaces and from these surfaces to the cucumber and chicken fillet slices, with the transfer rates varied from 20% to 100%. This study has highlighted the fact that pathogens remain viable on dry stainless steel surfaces and present a contamination hazard for considerable periods of time, dependent on the contamination levels and type of pathogen. Systematic studies on the risks of pathogen transfer associated with surface cleaning using contaminated sponges provide quantitative data from which a model of risks assessment in domestic setting could lead.

A2: There are two issue. Survival and recovery.

As expected bacterial spores > fungal spores > gram-positive bacteria > gram-negative bacteria

The recovery is typically less than 30%

A3: very correct- much depends on what you're trying to do with S/S "surfaces". If you're validating a VHP or peracetic acid vapor sterilization in an isolator, and using G. stearothermophilus, I'd expect close to 100% recovery on spores inoculated onto S/S. I'd expect less for fungal spores, MUCH less for GPC's and virtually NIL for GNR's, especially with any kind of drying time. They just won't stand it. Plus, if you're qualifying disinfection of surfaces, and looking for RECOVERY of percentages from those surfaces (with swabs, RODAC plates, etc.) after disinfectant/sterilant application, you'll be very lucky, in my opinion, to get 5%-10% recovery on non-sporeformers...... just doesn't happen, especially if you're using swabs or such for recovery- those are what I call questions you shouldn't ask because you won't like the answers.

That said, I don't know of any publications on the topic- maybe the results are too gruesome for anyone to publish them?

It'd help if we knew what you're going to be using the recovery studies for??

USP <1227> Validation of Microbial Recovery (you have 1000 CFU and dilute 2-fold to get 1 CFU?)

I was reading the new PMF newsletter on Antimicrobial Effectiveness Testing 51. And Reading USP <1227> cross referenced.

Does anyone know how you have 1000 CFU and dilute 2-fold to get 1 CFU?

USP <1227> "This validation may be performed by statistical comparison of estimated cfu from successive pairs in a dilution series. Prepare a suspension so that plating will provide approximately 1000 cfu per

plate, and then dilute twofold to a theoretical concentration of approximately 1 cfu per plate. Plate all dilutions in the series in duplicate, and incubate for recovery under the conditions of the Antimicrobial Effectiveness Testing 51 . Compare the estimates of cfu per mL from paired tubes in the dilution series by the formula:

A1: I am assuming by twofold, they meant halving dilutions, where your 1000 cfu is halved to 500 cfu, 250 cfu, 125 cfu, and so on until you approach 0.

A2: I guess that is poorly worded - I think it should more properly read:

"...1000 cfu per plate, and then dilute IN A twofold DILUTION SERIES to a theoretical concentration of approximately 1 cfu per plate."

1000, 500, 250, 125, 63, 31, 16, 8, 4, 2, 1 cfu/plate

Nice catch.

A3: There is a ready made solution to have 1000cfu. Its called Bioball (Lyophilised ball having precise number of microorganisms used for Antimicrobial efficacy testing) marketed by bioMerieux. This can be helpful for you. Follow this www.bioball.com link for more details.

Coliform

Anybody can clarify the difference between Enterobacteriacae and total Coliform .Is it ok to use the USP 2021 or USP 62 (test for Enterobacteria) for total coliform ? Is this a deviation?In your opinion the method USP 2021 or 62 (for enterobacter) can be an equivalent to method MFHPB-19 (for enumeration of coliforms).

A1: The coliform bacteria group consists of several genera of bacteria belonging to the family Enteriobacteriaceae Generally, coliform bacteria are generally classified as being facultative anaerobic, gram-negative, non-spore-forming bacilli that ferment lactose with gas and acid formation within 48 hours. I think that you would be better off in looking for Enteriobacteriaceae than just for coliform bacteria.

Acceptance criteria for microbiological quality

Has anyone considered this:

If we are able to accept a count of 200 when specified <100cfu/gm, 2,000 when specified <1000cfu/gm... "and so forth" ...then we are accepting an inherent error which we attribute to "hey, that's microbiology for ya!"

As professional microbiologists we are expected to keep this type of thing in the back (or the front) of our minds when reviewing all micro data, so then, how many of us consider this error factor when accepting a count of 99 cfu against a spec. of <100, or 990 against a spec. of <1000? The degree of error acknowledged means we should be reviewing these results as potentially huge, and that there might really be 190+ or +1900 cfu/gm in these samples. It is all too easy to accept an apparently conforming result, and to only apply the error factor as a "get out of jail free" clause in order to force release of an apparently failing batch. Due diligence should require us to at least note that this error factor is applicable to all count results, even those apparently passing "comfortably'. It does help when trying to explain to non-microbiologists how data from different tests on the same sample can be massively different , and yes, I know it's always very dangerous and really, irrelevant to do this, but bosses don't always "get" that TVAC of 80 and a BTGN of >100,<1000 can come from the same sample....

Of course, these results still pass specification when we are looking at data associated with contamination where the spec is an upper limit, but how about for a lower limit, using enormous numbers of probiotics?

There are those of us who have the dubious pleasure of assaying to a specification of Greater than 400billion cfu/gm; what would be the lower tolerance now? 200 billion??? 300 billion??? I think not; we have label claims to deal with! In order to answer some of the issues behind this problem we are developing protocols to define Accuracy, Precision and Robustness of the test method(s) which have traditionally been the domain of Chemists; quite a confronting idea for a biologist!

Has anyone got any suggestions as to how, for example, we generate a "Standard" cell suspension when the test we are trying to show Accuracy for is de facto the test we need to define the standard...a circular argument to start the whole thing seems like a problem from the outset.

A1: Interesting questions, mate.

When I took a Physics lab class at Victoria University of Wellington I remember having to give an error estimate when we made a measurement. It was good training.

Yes the compliance-mind would accept a batch with 99 cfu/g and reject it at 101 cfu/g. We must always consider the analytical limitation of the methods we use. In practice drug products have bioburdens well below the upper limits so if a batch had around 100 cfu/g it would be out-of-trend and should be investigated.

Yes probiotics would be a special case where the viable cell count is a measure of potency. The expectation would be that the labelled count would be present during the shelf life.

Ps. aeruginosa TSA vs. PCA

I need to qualify a new PW system. When I wrote my GPT SOP, I nominated Ps. aeruginosa (P.A.) as a GPT organism for PCA, as it is a legendary waterborne organism. As it turns out PCA does not seem to recover P.A. quantitatively as well as TSA, in fact not within the factor of 2 prescribed in GPT per USP <61>.

P.A. is conspicuously absent from GPT organisms for PCA manufactured by both Merck and BD/Difco.

Now there is wonderful controversy among my quality unit (none of whom have much microbiology experience); I contend that P.A. is not a suitable GPT organism for PCA- as counter-intuitive as that may seem - as implied by Merck and BD/Difco - and that it should be released with a planned deviation and the GPT SOP changed to advise use of the manufacturer's GPT organisms.

Has anyone come across this particular issue? Could an expert kindly offer some tuition about this as I am in the hot seat and could really use some help.

A1: Is PCA the only medium you are planning to use? In our water testing, we have found that R2A (low nutrient agar) yields significantly higher recovery for waterborne organisms as compared to TSA, although we are currently using both. Per USP <1231>, looks like PCA is considered a "high nutrient" agar, as is TSA. My analogy would be that trying to get good recovery from organisms that have been stranded in a low nutrient environment (purified water), then placing them on TSA is rather like taking a starving person to an all-you-can-eat buffet. May not be the best option. Rather than changing your GP organisms, it seems that you would more likely need a medium that can recover P. aeruginosa. If you cannot get this one to grow, the other "monas" organisms (didn't these all used to be Pseudomonads?) and those similar to them may not grow either and they are most likely to be in the water. I used to believe that TSA would grow everything, but having seen the comparisons in our data, I would never use it as the primary medium to qualify a new water system. Good luck with your qualification.

A2: I would not describe P. a as a legendary waterborne organism as it is rarely isolated from purified water systems. Perhaps you mean legendary like dragons and unicorns!

My preference was to use PCA and not R2A for water monitoring as it is often difficult to subculture isolates from R2A. This was noted in the 1985 paper by Reasoner and Geldreich. Also Reasoner (1990) demonstated that the highest colony counts were obtained using R2A spread plates at 28 degree C. This makes sense as molten agar reduces the count and 35 degree C is too high for waterborne bacteria

In general, the QC organisms recommended by the media manufacturers are inappropriate for pharmaceutical microbiology. Ralstonia pickettii would be a better choice.

A3: I am tempted to try and answer this but you need to decode some of the acronyms if you don't mind.

A4: My sympathies about being in the hot seat, I think as Microbiologists we all get to visit that from time to time!

You could look at your problem in a different way perhaps.

P.A. may not be a suitable GPT organism for PCA, maybe PCA is not a suitable medium for testing your new PW system. We've done studies and I know others have too - some extensive - to test which agar best suits their water systems. We test a few different systems here and have found that R2A gives the best recovery for our PW system and TSA is better for mains water. Possibly because, PW is low in nutrients so the organisms present in it prefer a low nutrient medium, where as mains water has a higher nutrient load so TSA works better, for us anyway. We tried PCA as well and for our systems the recovery was similar to TSA.

There's a really good article in one of the past PMF newsletters by T.Soli, which will be really useful for you. I'm sorry I can't remember which one.

Chin up!

A5: My sympathies about being in the hot seat, I think as Microbiologists we all get to visit that from time to time! You could look at your problem in a different way perhaps. P.A. may not be a suitable GPT organism for PCA, maybe PCA is not a suitable medium for testing your new PW system. We've done studies and I know others have too - some extensive - to test which agar best suits their water systems. We test a few different systems here and have found that R2A gives the best recovery for our PW system and TSA is better for mains water. Possibly because, PW is low in nutrients so the organisms present in it prefer a low nutrient medium, where as mains water has a higher nutrient load so TSA works better, for us anyway. We tried PCA as well and for our systems the recovery was similar to TSA. There's a really good article in one of the past PMF newsletters by T.Soli, which will be really useful for you. I'm sorry I can't remember which one. Chin up!

A6: You should be comparing the recovery on your new lot of PCA to that of a previously qualified lot of PCA using the same inoculum prep, - not to TSA. We don't have any issue with our BD media. Hope this helps.

bacterial endotoxins

i have one question regarding bacterial endotoxin test of cephalosporins

USP is not mentioned about the BET in MVC. USP saying that the test should be done only in MVD.

1. what is the difference between MVC and MVD
2. whether we have to perform the BET for cephalosporins in MVD or MVC is ther any guideline for MVC
3. how to decide whether the product has to be tested in MVC or MVD

Can anybody explain about the above queries?

A1: The terms Maximum Valid Dilution (MVD) and Minimum Valid Concentration are essentially the same thing, in that they express the amount of dilution of a product that can take place before a situation arises that any endotoxin present in the product will no longer be detectable because the sensitivity of the BET assay method in use been exceeded.

The difference is in the terms that the endotoxin level has to be expressed.

If the product is liquid, the endotoxin limit will be expressed as a function of volume e.g. EU/ml. In the case of liquid products, adding LAL reagent water to a product to overcome interference is expressed as a dilution factor and the limit to which the product can be diluted is defined as the Maximum Valid Dilution (MVD).

If the product starts as a solid form and has its endotoxin limit expressed as a function of weight e.g. EU/mg the dilution of the product after reconstitution is usually expressed as a reducing concentration e.g. fom 0.1 mg/ml to 0.01 mg/ml etc. and the limit the which the product can be diluted or have its concentration reduced, is defined as the Minimum Valid Concentration (MVC)

In fact in neither case, does the product have to be tested at the MVD or MVC. Provided the correct validation procedure has been carried out to overcome interference, the product can be routinely tested at any dilution or concentration that has been shown to be essentially free of interference up to and including the MVD or MVC. It is up to the manufacturer to develop a policy at to what will be the dilution or concentration of choice for their products. Often 1/2 the MVD or MVC is chosen as a useful norm.

A2: I will explain all your doubts with following example

Drug : Ceftriaxone Sodium Sterile 100 mg/mL
Endotoxin Limit : NMT 0.2 EU/mg
Lysate sensitivity : 0.125 EU/mL

MVD = Potency x E.L/ Lambda
MVD = 100mg/ mL X0.2 EU/mg /0.125EU/mL
MVD = 1:160
Letâ€™s find out the test concentration of Ceftriaxone sodium (100mg/ mL) at MVD (1:160)
i.e.100mg/mL / 160 = 0.625mg/mL

Letâ€™s find out MVC for the same drug
MVC = Lambda / EL
MVC = 0.125EU/mL / 0.2EU/mg
MVC = 0.625mg/mL

This ensures that the test conc. Of the product at MVD and MVC remains the same.

Answers
1. There is no difference between MVC and MVD; these are two sides of the same coin. In MVC you deal with product in terms of concentration and in MVD you deal with product in terms of dilutions. If you are a bulk manufacturer then if you fix the product concentration (Potency of product) letâ€™s say to 100mg/mL, then even you can use MVD method.
2. US FDA guideline 1987 Appendix D method II describes MVC method.
3. Itâ€™s your choice. If you want to test in terms of MVD then fix the product conc. (also mentioned in your SOP) and use MVD method. As explained earlier.

A3: Do you think this 1/2 dilution from the MVD may be chosen in order to take into account the 1/2 dilution of the sample that takes place in the mixture with the lysate in the reaction tube? In this case, a final dilution equal to the MVD would be considered.

A4: The sample is not judged to be diluted by the addition of the lysate, so this step does not need to be taken into account when calculating the degree of dilution.

A dilution of 1/2 MVD or 1/4 MVD for example, is often chosen because if a 'fail' result is obtained at the MVD, the batch is automatically a 'fail' unless the result can be shown conclusively to be due to a laboratory error. At 1/2 MVD, 1/4 MVD or less dilute, there is still a possibility for a retest and there is some warning that there is contamination occurring in the process before an automatic batch discard occurs.

A5: The dilution resulting from adding LAL to a sample is not considered, since the reagent is added to every tube in an assay. The standard method for performing the test accounts for this as the standards are treated the same way as the sample.

The reason that many people choose 1/2 MVD (or more) is so that they can set and detect alert and action limits at concentrations less than the endotoxin limit. If you test at the MVD you are conducting a pass/fail test at the limit. You will not know if there is a trend of increasing endotoxin until you get a test failure - regardless of test method. In actual fact, in photometric methods you may be able to detect increasing endotoxin below the limit (which is at lambda if you are testing at the MVD), but those will not be reportable valid data.

On a purely personal note, testing at half the MVD still does not give much warning of endotoxin concentrations approaching the limit, especially given the error of the test. I would generally recommend testing at 1/4 of the MVD.

A6: The object of endotoxin testing is to get the best valid result you can get, at the highest sensitivity you can get with your material of interest. Since many people don't have limitless time and resources to devote to experimenting and finding the "best" dilution for a valid endotoxin test on a given material, this process is often "short-cutted" by trying to validate at 1/2 and/or 1/4 MVD, and hoping for the best. Many products and materials, especially in the biotech arena, will interfere with the LAL test, unfortunately- so this is a hopeful way of finding a valid dilution level which will still give you some 'flex-room' below the MVD for your testing.

Several places I've been, we developed a standard 'screening' test for LAL pre-validation, where we tested a range of dilutions from undiluted to the MVD with a number of points in-between, and we would do this 'screen' on, ideally, 2-3 lots of material; this would give us a pretty solid idea where to look for the actual Guideline validation of the test-- or, in many cases, unfortunately, it would tell us we needed to go to the next level and start finding inventive ways to overcome inhibition which extended up to and well beyond the MVD.

GPT

Your answers are very appreciated,

1- Do you do controls (+ & -) for your media at the same time with your GPT (is this necessary to do ? if yes what argument you use to justify it and what would be the references)

2- Are you doing GPT for any media (solid & liquid) in duplicate? why? any references?

3- Is there any specification for room temperature(GMP-USP..)? what is the reference?

4- Any body can justify the use of flame under hood .I believe sometimes it is inevitable (while we know the flame can damage HEPA)?

A1: 1) It is necessary to assure the ability of the medium to growth, or to compare the counts. So you need to use a medium as a standard to determine the number of CFU added.

2) Yes.

3) The temp has to be comfortable for the operators. When incubators don't refrigerate, room temp has to be not more than 5ºC under the minimum of the work range. For example, for incubate at 30-35º, room temp must be not more than 25ºC.

4) If cabinet has vertical laminar flow, you can damage the filter. If you want to work with microorganisms in a cabinet with vertical LF, (biosecurity cabinets) you can use a loops incinerator, or to use disposable loops. But in my opinion the work with microorganisms under LF is not necessary.
If laminar flow is horizontal, you are not working with microorganisms, so, why do you want to use flame? The clean bench has to be installed in a classified room, according to the products you are going to test.

A2: 1) USP NF-online FAQ answered this with: "You do not have to test a previous batch in parallel. You can do the comparison 'on paper' if growth was clearly described."

2) The USP does not indicate that duplicate is required. It makes more sense to do single unit test for nutrient media using all necessary organisms, and duplicate with selective media for indicative and inhibitory organisms.

Still figuring it all out :)

A3: You're right. Duplicates are not required by USP. We perform the test in duplicates if medium is been used to counts. This is not required in the section "GPT" in chapter 61, but it is required in the section "recovery of microorganisms in the presence of product". There USP states: "For each of the microorganisms listed in Table 1, at least two Petri dishes are used".

A4: I am not sure I understand or agree with #1? How can you use counts on paper from some other media if the media under GPT was not inoculated with the same suspension of organism? Maybe I am missing what you are stating here. I see this as a matter of scientific principle to only have one variable. Seems like from what I have read companies are injecting 2 or more variables in their application of the growth promotion principles.

A5: It may be applicable for using ready to use suspensions where the reference counts are certified. For Inhouse suspensions one must carry out parallel reference media ie. previously passed media.

Fungus in water

Please any one tell me that according to pharmacopea why we not check fungus in drinking water or if we do what is references?

A1: Not aware that Pharmacopeia addresses drinking water other than to refer folks to regulatory (EPA) standards.

As one of the authors of the updated Section 9610 Detection of Fungi that will appear in the next edition of Standard Methods for Examination of Water and Wastewater, I had contacted a number of water treatment facilities - public and private - and learned that none of them routinely tested for fungi. They saw no reason, as drinking water has not been reported as a significant vehicle for fungal disease. When they did test, it was due to reports of off flavors/odors. That sais, they update will include methods and references.

Log reduction calculation reg

I am having a query regarding the log reduction calculation during the BI challenge study

The question is:

Consider,

"the inherent bio-burden level of the load is 300 CFU the population of the BI is 1X106 spores. After autoclave exposure the BI results as no growth. Now what will be the total log reduction of the load?”

I have two answers for this question

Answer 01
The Population of the BI is = 1000000
Bio-load of the load is = 300
Total bio-burden of the autoclave = 1000300
Log value of 1000300 is = 6.0001
Therefore
The total Log reduction is = 6.0001 LRV

Answer 02
The Population of the BI is = 1000000
Log value of 1000000 is = 6.00

Bio-load of the load is = 300
Log value of 300 is = 2.477

Total log reduction is = 6.00 + 2.477
= 8.477 LRV
What is the correct approach kindly explain?

A1: The total log reduction is 6. You started with 10>6 BI population, you achieved total kill = 6. The bioburden is not considered in this case.

A2: Answer 1 is correct. You can't add log values together in this way! (if you do, you are actually multiplying the values rather than adding them).

A3: If you started with a Log 6 BI and you killed it, all you know for sure is that you achieved a Log 6 reduction even though you may have achieved a higher lethality.

Inhibition of Staph. Aureus in Tryptic Soy broth in polyethylene containers?

We are experiencing varying frequencies of no growth of Staphylococcus aureus when performing Growth Promotion Test of Tryptic Soy broth in containers made of polyethylene. As we have never seen anything like that when the same media is tested in glass containers, it seems the problem is related to the container material. We are of course looking into leachables from the material, but have not found any inhibiting substances yet.

Is there anybody in this forum that have experienced similar problems and maybe found solutions to it?

A1: We do growth promotion of TSB fairly routinely in PET bottles and have never seen inhibition of S. aureus even when using low level (<100 cfu) inocula. Maybe try changing PET vendors?

Clean room classification standards and disagreements

While trying to get a better understanding of the rules and standards regarding classification I would really appreciate comments to the different standards and how they should be interpreted plus how they correspond or don't....

Does any one have a good reference to a comparative overview with connections between the different standards -plus comments to the questions below???

Is the FDA classification max limits "in-process" measurements (does dynamic equals with human activity") and not "at-rest" (room ready for production)??

If the FDA limits are for "in-process" -what specified air-cleanliness levels is then recommend for the "at-rest" occupancy state in order to meet "in operation" conditions?

How is the disagreement between the FDA and the EU CGMP to be explained and interpreted?? -looks like the "In-process" FDA, but how should the disagreement with the EU CGMP then be interpreted, because then the FDA "in process" correspond to the limits "at-rest" for the EU CGMP?

Do the ISO 14644 classification vary depending on the occupancy state of the room?? -does this mean that a room can have 2-3 different ISO classes depending on the activity/state (as-built, at-rest or in-process)?

Why do the EU GMP doesn't have corresponding correlations between the limits for "at-rest" and "in-process" specifications -where there for class A is no difference; B has a factor 100 and C only a factor 10??

Is classification and/or re-classification generally recommended performed during "in-process" or "at-rest"?? -"in-process" provide a good, realistic, relevant and worst case situation but on the other hand "at-rest" will provide a more clear measurement of the room-class not affected by variation factors like room activity......

Is re-classification measurements per sample location repeated - how many times (just 1, 2 days or over 3 days)?? -in case 2 or 3 should it then be days connected or different days with some time in between??

and last -Do anybody have issues with fine working but "older" models of particle counters having difficulties passing the ISO 21501 calibration -and how is it recommended to handle the deviations if they fail??

-Can failure on parameters not in use be temporally ignored (eg. counting efficiency 50% for 0.3ym particles, when only 0,5 and 5.0 ym is used..)?

validity and stability of microbiological results

I have a doubt regarding how microbiological results can be used throughout time, that is to say, the validity and stability of this results. For example, imagine that results obtained in March 2010 were: TAMC: NMT 1000 cfu/g, TYMC: NMT 100 cfu/g and absence of pathogens/g. For how long can I use these results? I mean, when it will be necessary to analyse again the product to determine its microbiological quality? Are stability studies required?

A1: The microbial results whatever you have obtained stands good up to the retest period whatever you have fixed for the material(Usually around 2 yrs).

If at all there is any change in the storage conditions or any packing defects are found during storage period, you will have to reconfirm the microbial status.

A2: Absent spontaneous generation and assuming physical/barrier (package) protection from external microbial contamination - you have the same concerns you'd have at the time of the test - level of detection both statistical and spatial and potential growth of undetected bugs. The latter is better addressed through elimination by process (e.g. hygiene or even terminal sterilization) and prevention by formula (e.g. preservation) controls.

A3: Question is not clear. Please mention the product about which is question is about -waters or raw materials or finish product or others.

A4: If you are claiming that your product stays a certain way throughout its shelf life then it is recommended that you perform stability studies to support this claim and the dating on your product's shelf life.

A5: It depends on your product ; if it is a powder there are no chances of further enumeration and increase in the value of the results. If your product is a liquid then there is a chance that the result shall change more rapidly. If your product is a liquid and contains a preservative the results will not change much till the effectiveness of preservative remains. Hence, based on the product you have to study microbial load at frequent intervals say every month till the expiry of first three commercial batches; Based on the trend you can decide the frequency.

BET testing alternatives

I am encountering back order issues with our BET (Bacterial Endotoxin Testing) supplier. They have recommended an alternative method to BET testing besides using Limulus amebocyte lysate (LAL). Called the PyroGene® Recombinant Factor C Endotoxin Detection System by LONZA.

Have you all any experience with it?
Is this an acceptable alternative test to BET by the USP, EP.... or regulatory bodies like the FDA, EU, TPD…?

A1: An interesting proposition. While I cannot speak to point 1, I can address point number 2. PyroGene® Recombinant Factor C is not recognized by USP, EP, or JP. The harmonized compendia from each of these bodies specifically state that LAL is prepared from amebocytes obtained from Limulus polyphemus or Tachypleus tridentatus horseshoe crab species. As a recombinant single enzyme product, PyroGene® Factor C is from neither species; therefore it is not LAL and it is not a BET test. Additionally, it is not licensed by CBER. This has profound significance. It is very likely that your regulatory fillings state that WFI testing (and all relevant in-process and final product release testing) will use a BET method. As previously stated, PyroGene® Recombinant Factor C is not a BET method. PyroGene® can certainly be validated but, in addition to the validation effort, your regulatory submissions may require an amendment to reflect the use of a non-BET test. Not to do so presents a significant regulatory risk.

A2: I was surprised to hear that you have had problems sourcing LAL reagents from your supplier. You don't say who your supplier is but I noted you mention Lonza at the end of your mail. If Lonza is your current supplier, I can state there are no issues with LAL based endotoxin testing reagent supplies
If you refer to a recent letter sent around by Lonza, this is not meant to imply a problem of supply of LAL reagent but suggesting a way forward in the future when demand outstrips supply. As a biologically sourced reagent from a protected species, this time will undoubtedly come as demand continually increases; at which point alternative methods will become more valuable.

As an alternative to LAL based methods Pyrogene works very well and consider this. The Kinetic Methods that are in common use today were not always in the USP EP or JP, but that did not stop them from being taken on board by many leading pharma companies.

Call your supplier and see what they say.

A3: Just thought I'd ask a question regarding the LAL BET and the inevitable constraints of meeting the increasing demand.

Is it possible to culture in vitro the cells or tissues which generate amebocytes?

A4: While trying to get a better understanding of the demands and standards regarding classification, re-classification and compliance testing I would really appreciate any comments to the different standards and how they should be interpreted plus how they correspond or don't....

Does any one have a good reference to a comparative overview with connections between the different standards -plus comments to the questions below???

Classification limits The FDA limits are for "in-process" -what specified air-cleanliness levels is then recommend for the "at-rest" occupancy state in order to meet "in operation" conditions?

How is the disagreement between the FDA and the EU CGMP to be explained and interpreted?? -looks like the "In-process" FDA, but how should the disagreement with the EU CGMP then be interpreted, because then the FDA "in process" correspond to the limits "at-rest" for the EU CGMP?

Why do the EU GMP doesn't have corresponding correlations between the limits for "at-rest" and "in-process" specifications -where there for class A is no difference; B has a factor 100 and C only a factor 10??

The Compliance testing

What occupancy is relevant for classification and/or re-classification "as-built" or "in-process" or "at-rest"?? -"in-process" provide a good, realistic, relevant and worst case situation but on the other hand "at-rest" will provide a more clear measurement of the room-class not affected by variation factors like room activity...... and as built tells you about the construction..

Can you distinguise between re-classification and compliance testing for both meaning and the testing needed -if compliance testing is defined at the regurally check for compliance according to ISO 14644-2 table 1 and re-qualification is the process to demonstrate reestablishment or preserved compliance after the integrity to some significant degree has been affected or/and the room may be downgraded for a period..

Is re-classification measurements per sample location repeated - how many times (just 1, 2 days or over 3 days)?? -in case 2 or 3 should it then be days connected or different days with some time in between??

and last -Do anybody have issues with fine working but "older" models of particle counters having difficulties passing the ISO 21501 calibration -and how is it recommended to handle the deviations if they fail??

-Can failure on parameters not in use be temporally ignored (eg. counting efficiency 50% for 0.3ym particles, when only 0,5 and 5.0 ym is used..)?

A4: I suspect that any replacement will need full validation and probably submission of a change request to the regulatory agencies concerned. I doubt that different tests can be considered to be ‘equivalent'.

A5: Hi, All, From what I understand, the recombinant test is not yet licensed by FDA as a BET and/or alternative to the existing BET. FILED with FDA, yes, but not yet approved. Whether or not it's approved, it would require full Guidelines validation with your product(s) and prior-approval submission to regulatory agencies before use. M. [log in to unmask]   I suspect that any replacement will need full validation and probably submission of a change request to the regulatory agencies concerned. I doubt that different tests can be considered to be ‘equivalent'.

A6: It may prove beneficial to ground truth any statements you hear about the supply of LAL raw material. Credible data to support claims of eminent disaster on an industry-wide basis are surprisingly rare for an event that would have such a major impact on industry. Why might there be such a lack of data? Have you seen any vendor refuse an order because raw material is no longer available? When reality and sales literature do not synch, the high probability choice is to go with reality.

A7: Whilst I don't believe that the PMF forum is the proper place to debate the merits of the PyroGene assay(r), I do feel obliged to correct inaccurate information that appears in these email threads.

There is no regulatory significance in the fact that the recombinant Factor C assay is not licensed. The only reason that LAL reagents are licensed is because they originate from the blood of the horseshoe crab and the these reagents are still classified as 'blood products'. Licensing has nothing whatsoever to do with their suitability to perform the Bacterial Endotoxins test (BET). PyroGene was approved for sale several years ago and prior to its release, we specifically asked the FDA if this product required a license. The answer was 'No' because it is not derived from blood as a raw material starting point.

A8: I like the way you are thinking. In theory this would mean there would be a steady stream of cultured amoebocytes from which the lysate could be removed and turned into the LAL reagents we all know.
The biggest problem would be, in order to make this continuous the amoebocytes would need to be transformed genetically, to allow continuous cellular division to allow constant generation of lysate.

The successful research would then have the same problem as those generating rFC and that is it would no longer be LAL or TAL as per the definitions in USP and EP and JP.

Actually, rather than have amoebocyte cells producing the LAL in culture and therefore keeping the secondary pathway intact to generate false positives, why not have a cell that produces the factor (Factor C) that responds to endotoxin alone.
This is already done and is referred to in [name redacted] original e mail

A9: According to the "2010 Review of the 2009 ASMFC Fishery Management Plan for Horseshoe crab (Limulus polyphemus) "the stock assessment indicates abundance has increased in the Southeast and Delaware Bay Regions "Any suggestion that LAL demand outstrips supply is not true. Dr. James Cooper, co-founder of Endosafe worked with the South Carolina fisheries and game conservation committee to have the specie protected. Horseshoe crabs have existed for 400 million years. The ASMFC management plan will ensure that they will continue to flourish for at least another millennium or so.

A10: It is not the LAL demand that is decreasing the supply of horseshoe crabs.

The Baymen and fishermen in these areas use the horseshoe crab meat as bait. Until there is plan in place to teach the fishermen not to over-harvest, the horseshoe crabs will continue to be in trouble.

Several conservation groups are trying to do just this. With the exception of New Jersey, conservation groups in other states have been unsuccessful.

A11: I am not familiar with the rFC term but I gather it is related to the standardization and acceptance by regulatory bodies such as the BSI and the FDA and other pharmacopeia. Are horseshoe crab amebocytes the only amebocytes that contain these clotting factors? I know Dr. Bang and other hand done the foundation research with horseshoe crabs but have there been efforts to 'bioprospect' other invertebrates such as snails. I know they have amebocyte producing organs (APOs) which protect against disease by parasites and they are easily raised and are quite prolific. Perhaps it's already been done I'm not sure.

The issue of standardization would have to be met with validation data to prove that the performance of the alternative amebocyte lysate is at least equivalent in performance to LAL. It would take some time but I think competition is good for business.

The issue with transforming the amebocytes in order to make them a continuous cell line I am sure can be overcome. I've seen a recent publication (2010) where the primary culture of snail APO was recorded. http://memorias.ioc.fiocruz.br/101%286%29/5582.pdf A similar approach may work for horseshoe crab amebocytes.

To clone the factor into a cell line, bacterium or yeast may not yield all the true features of the clotting which as you pointed out would not be a bad thing. The problem might then be purification of the factor.

A12: rFC is recombinant factor C. Factor C is that part of a LAL reagent that detects the presence of endotoxin and triggers the cascade that leads to either the gel clot or the other end points of turbidity or chromophore release.

It is produced in a transformed cell line and incorporated into an endotoxin testing kit that is available on the market.

Comparative validation work against a pharmacopeial method has already been completed and published.

A13: The "2010 Review of the 2009 ASMFC Fishery Management Plan for Horseshoe crab (Limulus polyphemus)" provides some quite useful information, particularly table 2 "Reported commercial horseshoe crab bait landings by jurisdiction. Showing that conservation efforts have been effective in several areas. Most notably South Carolina has 0 reported landings since 1999, Georgia has 0 reported landings since 2000. Efforts in New Jersey resulted in a massive reduction in landings from 2005 to 2006 and since 2007 New Jersey has had 0 reported landings. South Carolina in particular has recognized the significance of the horseshoe crab. I think the numbers show that crab population particularly in the Southeast will provide a reliable source of raw material for LAL for quite some time.

finished dosage form

The term finished dosage form is used in USP<1111> . Is this interpreted as testing micro on finished packaged form or is this interpreted as testing on any forms of finished product either in a bulk form or a finished packaged form?

USP <51> specifically uses the term finished packaged form which is very clear that AET should be done on the final packaged product.

Product bioburden

Can any one clarify me regarding the product bioburden failure?

If at all the product bioburden fails and if all the environmental sampling and the analytical results are OK, Is it required to take the additional samples for the sterility?(because the bulk solution will not be available for the repeat analysis). If answer is NO how to conclude the failure?

A1: Please explain how a product bioburden can fail???

A2: Shall we first and foremost think seriously about the root causes of bioburden before going deeper into that issue. Could you please give me some ideas or knowledge about the principle relating with Good Clinical Practice or GMP.

The issue is "A Technician infected with Hepatitis B virus, Hepatitis B career, can be qualified to do a clinical job or doing doing a laboratory job in the Health Laboratory or in the Sterile products manufacturing Unit." For this moment, we don’t have any idea to disqualify or transfer to another chemical lab.

A3: BULK BIOBURDEN IS NOT A CONCLUDING TEST FOR REJECTION OF ANY PREPARATION. ADDITIONAL SAMPLES ARE NOT REQUIRED AS LONG AS YOUR MEMBRANE INTEGRITY TEST BEFORE AND AFTER FILTRATION OF THE PRODUCT IS OK

A4: When your product contains more bioload or exceeding the limits of your product specification you need to go for investigation.
You need to check personnel monitoring results.
Post bubble test.
Identification of organisms.

There is no requirement of taking additional samples for sterility.
Batch need to hold for release unless your investigation closed.

PET testing

I have a few questions on PET testing again (thanks to those who answered my last lot of questions it was very helpful).

In the European Pharmacopeia in the section Efficacy of Antimicrobial Preservation in the section detailed "Preparation of Inoculum" the EP states that to harvest the bacterial and C. albicans cultures, use a sterile suspending fluid, containing 9g/l of sodium chloride R. My question is what is an acceptable suspending fluid for this? I have been looking at ¼ strength ringers but that only contains 2.25g/l sodium chloride and what does the R stand for?

Also I have an emulsifying ointment for PET testing, It is a solid wax. How do I test this? Can I reconstitute it in something?

And last but not least I want to set my pass/fail criteria for validation of PET. What are acceptable limits?

A1: If I recall correctly, the R is simply referring to Reagent Grade Sodium Chloride; if you turn to the Reagents section of the EP Monograph, there should be a thorough description of "sodium chloride" there. Unfortunately I do not have access to the EP right now to verify it, so my apologies in advance if I am wrong!

A2: R stands for 'reagent' and this is defined in the pharmacopoeia and you should use the defined material. In this case there should be a known level of endotoxin in the diluent. 9 g per litre is equivalent to human plasma sodium chloride content.

A3: Hello Isabel - the suspending fluid is normal saline - so just water and the 9 g of salt. In my old company quite a few years ago we used to test chap stick like formulations. What we used to do is to soften the formulation by putting it at 37C for about maybe 1/2 hour prior to inoculation, and followed with a very good mixing using a pipette. We also softened it at the same temperature prior to any sampling, and mixed it really well prior to each sampling (with a pipette). This was in the 70's and there may be better methods now. We did find an article where they used some chemical to soften the formulations, but we found that this chemical promoted growth of organisms, and we were getting false positive results.

To validate the PET you really just need to validate your neutralizing medium, and there are specific procedures and criteria for those in both pharmacopoeias I believe (US and EP).

I don't know what the R stands for. In the USP is says normal saline TS - where the TS stands for Test Solution. It may be something similar in the EP.

A4: In the European Pharmacopeia in the section Efficacy of Antimicrobial Preservation in the section detailed "Preparation of Inoculum" the EP states that to harvest the bacterial and C. albicans cultures, use a sterile suspending fluid, containing 9g/l of sodium chloride R. My question is what is an acceptable suspending fluid for this? I have been looking at ¼ strength ringers but that only contains 2.25g/l sodium chloride and what does the R stand for?

A: you can use sterile 0.9% Nacl solution . R stands for Reagent mentioned in the EP.

Also I have an emulsifying ointment for PET testing, It is a solid wax. How do I test this? Can I reconstitute it in something?

A: use sterile solvent which can dissolve the product .

And last but not least I want to set my pass/fail criteria for validation of PET. What are acceptable limits?

A; Pass shall be no growth during the observation period.

A5: I think Isabel was asking after a means to facilitate challenge testing of the ointment - rather than procedure directed at challenge testing.

PET testing of a material dissolved in a solvent is technically irrelevant and probably misleading regarding the contamination risk PET is meant to indicate - unless that is how the product will be used.

A6: I have a fundamental question: Why would you want to do a PET on a non-aqueous product? This should not support microbial growth and there would be all sorts of problems in actually undertaking the test.

With respect to the question of setting pass/fail criteria: If you are using the pharmacopoeial test why would you not apply the pharmacopoeial requirements?

A7: Contriving a way to conduct a PET that has no relevance to the product is useless.

Inhibitory property testing of media

For testing inhibitory properties of media, I think it would be most appropriate to inoculate a released batch of nutrient media (eg: TSA) with the same inoculum of the inhibitory organism used for the selective media and compare, but what would the quantitative difference be between them if any?

A1: For inhibitory properties one must use near to >100 CFU per selective broth/agar and parallely confirming the count on TSA if desired.

preincubation of media

One of my colleagues just attended a class about Sterility Testing. During that class, several people mentioned that they were preincubating media that is used for environmental monitoring of isolators and clean rooms, due to "false positive" results in the aseptic areas. My question is directed at those who have instituted this type of procedure. Has it truly led to decreased positive results in the aseptic areas? Also, if this is being done, what is the approximate contamination rate of pre-purchased media?

P. fluroscence

I `m looking for a method to detect Pseudomonas fluroscence (can we use USP 62).

A1: Here is a published paper (full-text) in AEM that might be useful.

http://aem.asm.org/cgi/reprint/26/2/219.pdf

Brodsky, M. H., Nixon, M. C.
Rapid Method for Detection of Pseudomonas aeruginosa on MacConkey Agar
Under Ultraviolet Light
Appl. Environ. Microbiol. 1973 26: 219-220

Preservative assay test by Microbial Assay method

Sterile ophthalmic combined products containing antibiotics and steroid are used a preservative due to multi dosage forms. Is it possible to measure preservative amount by Microbial Assay method in that product and how is possible to perform PET for that product?

A1: I don't think you can measure the amount of preservative in the product microbiologically. If a previously passing test starts failing, then you can assume that the concentration of preservative has gone down, but you cannot establish an exact level. There are some tests that can be done - called titration to failure - where you add various concentrations of the preservative from 0 to above your use concentration. The results will give you some idea at which level the formula will fail the PET, but again, the correlation tends to be poor, and not always reliable. You will need to establish the levels using chemical assays as far as I know.

For testing methods - you can melt the ointment at 37C prior to inoculation and prior to sampling (see my last post). But in between samplings, keep the inoculated samples at the 20-25C that is in he pharmacopeias.

penase concentration

i have one question regarding inactivation of cephalosporin antibiotics

can any body say how much quantity of penase is required to inactivate how much concentration of antibiotic
is the penase that is added in the media for environmental monitoring is active longer period if yes what is the period

Cleaning validation

Kindly suggest any guideline or pharmacopoeia ref about pathogen testing in cleaning validation of equipment it is mandatory, because most of pharmaceutical groups carried out only testing of total aerobic microbial count from cleaning validation sample (Swab /Rinse).

A1: I am curious to comment on this topic. The factors that affect the bioburden of equipment surfaces are quality of water being used for cleaning and final rinse the cleaning procedure and the final air drying If compressed air is used for drying, the qualify of the air the quality of the environment in which the equipment is located the time period the clean equipment is held the procedure for control like if there is any cleaning further before use

A comprehensive monitoring plan should be in place to understand the quality of the above sources. If one has the effective procedure to differentiate and identify the objectionable organisms from all the above sources, then performing the test for specified microorganisms (as part of cleaning validation)may not be required and my not add any further value. The industry performs the 'test for specified microorganisms' as part of cleaning validation to demonstrate that the the equipment surfaces are at least free from specified microorganisms. Hope this gives some clarity.

unloading autoclave in aseptic area

We sterile-fill parenteral products that can't be terminally sterilized. I'm wondering how the rest of industry views the practice of unloading an autoclave that is in a Grade A space adjacent to the filling line. When our operators unload the autoclave, their gowns can become damp from the steam. Have any of you found any link between damp gowns and an increase in gowning hits?

Identification

1- As you know in USP 2022 there are all details for identification of all pathogens(if applicable) but not in USP 62 (it says only to identify). could we use USP 2022 as a reference for identification for USP 62 ? If not how do we do the identification for the pathogens (S.a, P.a, E.c, Salmonella)? Is there any other references? Are the identifications in USP 2022 enough for detecting the pathogens ? Should we do API

automatically (or we do upon request)? Is there any other references in USP for characteristic colonies description of pathogens (except USP 62 and)?

2-If we have a validated autoclave , do we need to do BI (biological indicator) on a regular basis (example: weekly or monthly) ? if yes what would be the frequency and how we can answer to an auditor asking why you control your validated autoclave? any reference you recommend? what if the autoclave is not validated (the cycles)?

3- If we have OOS result for a pathogen(presence of patho) and the result of retesting(requested by client) shows the absence, how we explain this and do we need to complete again the OOS report (a auditor is asking for this)?

4- If the temperature of an equipment (eg.incubator) is not within the range after the first reading and it goes back to normal after 1 hour of observation (second reading) do we need to do an investigation or we can accept the result by adding a note on result sheet(or log book).

5- Could we use USP 62 for detection of P.fluorescence, if yes do we need a deviation report? if not do you recommend any reference?

6- Is there any reference which explains the interval of test for challenge test(2,7, 14, 28 days) I mean these intervals are based on what kind of studies?

A1: 1) You can identify through API or another system.

2) BI are required. You need to establish the frequency. We test with BI once a week, and daily with Thermalog´s. The validation is performed in general once a year, it is a very long period. The cycles have to be validated.

3) If a pathogen had been detected, the product has to be rejected.

4) I suspect that you are not continuously recordring the temperatures. I don't know if the temperature was under or upper the range. You need to do an investigation. Evaluate the risk and take a decision according the investigation.

A2: Regarding point 4 .The excursion in temperature shall not occur unless and until there is a change in setting (temperature knob) .Power failures shall be immediately backed up by generator with in 1 minute . Hence, ensure that whether the setting (of temperature) changed while cleaning or any other operation. If there is change due to this reason, one need not worry about the result.
If the change in temperature is due to malfunctioning of any part of the incubator , the things shall be immediately put in another incubator with the required temperature settings till the malfunctioning is corrected and checked for correctness.

H202 - VHP (mg /L - ppm)

I have a few questions regarding Sterilants for SIP.

1: If a Functional Design Spec for a SIP Process states an upper limit of 116mg of 35% Vaporised H202 / L of air what does this mean is terms of
ppm H202?
I have tried some calculations but came up with a figure of just over 29,000 ppm. This seems an inordinately high value to be used for decontamination purposes. I usually see values quoted in the 400 - 3000 ppm range. Am I missing something?

For purposes of this discussion one should assume 100% vaporisation efficiency (in reality I believe this will be influenced by evaporation temperature and humidity as well as downstream / contact surface humidity and temperature conditions etc).

2: Can anyone recommend a good General reference for Hydrogen Peroxide & VHP as a decontamination agent.

3: Can anyone recommend a good General reference for Peracetic Acid Liquid & Gaseous as decontamination agent.

A1: There is a book out there that may help.

Title: Advanced Aseptic Processing Technology
Edited by James Agalloco and James Akers
Published by Informa Healthcare.

This book as good information about VPHP decontamination along with other methods including chlorine dioxide gas.

A2: I am not clear about "Vaporised" term (116mg of 35% Vaporised H202 / L of air).

By ignoring this term, "ppm" shall be calculated as follows,

116mg of 35% H202 / L

= 116 x 35/100 mg of H_2O_2 / L

= 40.6 mg of H_2O_2 / L

= ~ 40 mg of H_2O_2 / L

= 40 ppm of H_2O_2 ("mg /lit" is equal to "ppm" terminology)

Therefore,

116mg of 35% H202 / L = ~40 ppm of H_2O_2

A3: 116 mg/liter of H_2O_2 in air (0% RH) would require a temperature of over 100C to keep the gas from condensing. At this temperature and assuming you are at 1 atmosphere, the concentration in ppm would be well over 100,000. I would not recommend trying this as you would be approaching explosive levels. I would also not recommend VHP for any SIP application due to its' poor penetrating capabilities. There is a free reference library on-line for VHP at www.isolationinfo.com.

Biological Indicators for Peracetic Acid: D-value

I'm looking for a contract lab that can do D-value determination on biological indicators of G. stearothermophilus with vaporized peracetic acid. Any suggestions?

A1: I believe Raven Labs might be able to do this for you, but don't quote me on that.

A2: NAMSA can perform this testing.

N2 Bioburden / sterility testing reg

This is regarding the N2 gas bioburden & sterility testing method.

We are doing the N2 gas bioburden & sterility testing by inline filter holder method. Where we will sterilize the inline filter holder (of 47mm dia) with 47mm, 0.45µm - PVDF membrane by autoclaving & after that we will collect the N2 sample by filtering the N2 through it. During the sampling the flow is measured by a flow meter which will be attached at the outlet of the inline filter holder, we maintain the flow of 100L / minute. Then the filter membrane will be placed in a SCDA & SDA media (in case of bioburden) & SCDM & FTG media (in case of Sterility test) under grade A LAF for recovery of microorganisms. The positive control is made by inoculating 10 – 100CFU of the test organism over the same quantity of N2 sampled membrane by spread plat method.

The question here is whether this method is correct method or we need to do the sampling by bubbling in a sterile 0.9% saline solution & the sampled saline shall be test for bioburden / sterility.

CIP bioburden cleaning validation (including surface testing)

Does someone have any experience doing CIP bioburden cleaning validations? Any suggestion where to find information? So far they are two approaches found to spike a product with one of the residents and run through the line, appropriate holding time, then clean and sanitize, run a clean second product and collect the product from the line for bioburden testing in addition collect surface samples from specific critical point throughout the system for surface testing.

TOC Analyser

I have a question regarding Total organic carbon analyzer(Shimazdu/any other), Is it require the system suitability calibration daily/weekly? If weekly, then how can I justify instrument is ok for one week. Is there any validation for this?

A1: Your instrument should go through a thorough qualification. Your instrument supplier should have data to supply to you on the frequency of system suitability testing requirements. At the same time, you will gather data on your individual instrument. You will trend this data. The frequency of performance will be more frequent initially as you gather trending data to support decisions on future frequency. You may also review the ability to perform a single system suitability test in-house to verify instrument performance. The instructions to make these standards should be in your instrument users manual.

I understand the high cost of these standards.

A2: If you are speaking of a benchtop unit, I would recommend that you do system suitability every time that you use it. I treat it as if it were any other analytical instrument.

A3: If you want to carry out your system suitability on weekly basis then you have to collect the data firstly by performing the system suitability on daily basis which will cover the stability of your system suitability stock solutions and the response of the instrument for this at least three consecutive stds. Data will be required and this whole study will be based on a protocol which will cover the scope and stability of the std. solution

if you are going like that firstly preparing the 500ppm stock and further diluting it for daily 500ppb std then the protocol will cover that the solution 500ppm is stable for one month or how long you want to store the scope of storage is that with one std system supposed to give same response if it is working properly.

So capture the data for three stocks and u are able to reduce the system suitability frequency from daily to weekly basis but there is one condition that there will be no failure during the study due to the performance of the system

Analytical error will be acceptable with proper investigation and justification

And one more thing store the stocks at 2 to 80C positively and remove before further dilution only(let them come to normal temperature before diluting) and store 1,4 Benzoquinone protected from light.

A4: I wanted to chime in on this subject after reading a couple responses and give you a better idea of what you need to be thinking about as you consider this question.

From USP: ?The suitability of the apparatus must be periodically demonstrated as described below.?

So the question is, what is meant by "periodically?" The trick is, only you can answer the question because it's a risk-reward situation. I have customers on one end of the spectrum like Chris, who believe that running a system suitability test (SST) every time they run the instrument means that even if they fail the test and investigate the instrument to find it out of calibration or something, they will have so little exposure in the way of questionable product that it is worth the investment - this decision is conservative but completely sound and very low risk, which from a product safety standpoint is something we should be striving for.

Now devil's advocate position. While product safety should be number one, you have to weigh the benefit of frequency versus the "cost" of time and chemicals. If you or someone in your organization decides you need to be conscious of the cost as it relates to SSTs for the TOC, and that they should be minimized if possible, then you have to be methodical about your approach.

On the other end of my customer spectrum I have folks who perform the test monthly. Now I personally think it is silly to "save" money buy reducing the frequency to monthly, and I say that because of the significant risk you take on in doing so. As an example, if you run your SST on the 1st of every month, and on the 20th of the month fail an initial SST and a recheck, you have a problem on your hands. You now have to do some investigating to demonstrate you know when the instrument went out of conformance. Your data, your water and your product is potentially all in question - and in this case, it's 19 days worth!

In the event of a failure with Chris, only a few hours worth of data/water/product is going to be in question. I think most everyone would agree that they would rather be responsible for investigating and defending a few hours as compared to a few weeks.

So as you construct your own individual risk-reward scenario, think about the potential situations you may encounter. [names redacted] both laid out the approach you would take in defending decisions to widen the gaps between each SST. You need to take a lot of data initially, so that you can defend statistically your decision to run it less frequently - weekly or whatever. Your logic would be to support the statement that: relative to the risk you are willing to accept, there is no additional benefit to running the SST with each run, or daily or whatever.

Like most quality-related decisions, risk-based decisions are a great approach. Hope this helps you appreciate the impact of the decision a little more.

Regarding <1111> MLT

regarding to USP 2009 in chapter <1111> for microbiological examination of nonsterile product the acceptance criteria in table 1. page 605

101 cfu: maximum acceptance count = 20;

102 cfu: maximum acceptance count = 200;

103 cfu: maximum acceptance count = 2000;

I got confusing .

for example: 102 (200) result limit, each sample will has replicate plate

in 1st plate 120 cfu/g and

in 2nd plate 130 cfu/g so the total count = 250 cfu/g is it over limit

Or it's mean the average of the plates 125 cfu/g ? and it's under limit.

A1: Whenever there are two plates used for the same test, the average is used to produce results.

A2: In your example the average (125 cfu/g) is over the limit (the limit is 100, not 200), but after an adequate investigation maybe you could conclude that the product could be released because the count is not more than 200.

A3: If you are plating in duplicate, which is what I surmise, then you would perform the latter with an average of 125 cfu/g.

A4: As I remember, EU says to multiply the limit with 5, ie ,10^2 becomes 500 and so on.

A5: USP, Eur Ph and JP Pharmacopeias have been harmonized, and now factor is 2.

A6: Ph Eur has harmonised with USP and JP so that the maximum acceptable count is 200 not as previously 500.

Regarding 5% Dextrose

5% dextrose solution will support the Organism growth ? in this matter is there any guideline please suggest me.

A1: Beware of osmophile yeasts & moulds. If you want to be sure - verify Aw-value.

A2: Think you'd need a much higher glucose concentration before worrying about osmophiles.

rotating surface samples

Does anyone know of any reg's regarding the rotation of surface samples in ISO 7 clean rooms? My client, in question, is marketing to Europe as well as the US. I haven't seen anything in the USP that talks about this and I don't remember seeing anything like this in ISO 14644. Right now, we are sampling 86 locations in the clean room each session and the client would like to cut back on their overall costs and sample 43 locations each quarter on a rotating schedule. So, I have been asked to provide regulations, if applicable, on this matter. They don't want to make any changes unless they can back it up with regulatory documentation.

Media Water Activity

Can anybody recommend a system to measure the Aw of prepared media in plates? Also what values would be expected to give good performance or alternatively when growth promotion may be adversely affected?

A1: I have a question as to why you would want to determine the Aw of microbial growth media in plates. Because media is made with water, I would suspect that you would obtain a Aw reading of 1.0. Water Activity (Aw) of a sample measures the potential of microorganisms to proliferate. I would think that growth promotion and pH of prepared media be more appropriate as a QC test. If the prepared media doesn't support the growth of microorganisms in your growth promotion test, I would not use it.

A2: I want to believe that you are working with certain microorganisms that require specific water activity range for optimum/maximum growth.Such organisms as Yeasts, fungi or Bacteria.In that case, my advice is that you look for an Equipment called AQUALab 3 series (produced by a Company by the same name in US),With this instrument you will be able to measure the water activity of your medium before or after autoclaving. Depending on the type of organisms being cultured, you require a reading of between 0.98 - 0.93 for yeasts, 0.1 - 0.83 for Bacteria and 0.99-0.81 for fungi.Although slight differences will occur where aw is modified with particular ionic or non-ionic solutes This is the easiest way to measure aw. Relative humidity measurement and subsequent conversions applies too.This is more rigorous.

A3: Please check the accuracy in your message "0.98 - 0.93 for yeasts, 0.1 - 0.83 for Bacteria? and 0.99-0.81 for fungi?".

A4: I recommend that you purchase the book "The Application of Water Activity Determination in the Pharmaceutical Industry" I co-edited with Anthony Fontana. It can be ordered from the website www.pda.org

The Aw ranges cited for bacteria, yeast and mold is not correct.

Influence of temperature on probiotics

The point is that we have two probiotic Ginilac and Entenorm (their consitent Lactobacillus acidophilus, Lactobacillus bifidus, Stereptococcus thermophilus, Lactobacillus delbureki subsp. bulgaricus). At list time we've discovered that storage temperatures was abolished. Of course this became reason the derogation effectiveness of product because unfavorable condition became reason of death of microorganisms. I explained that fact but they want for me scientific explanation of this process, which processes are goinig on the level of cell . Also how much irreversible is this process.

Neutralization of Silver Sulphadiazine

In pharmacopoeia is stated as below for Silver sulphadiazine Cream : Microbial Count does not exceed 23 cfu per gm in absence of E. coli, Salmonella, St. aureus and Ps. aeruginosa. We know that Silver sulphadiazine is showed inhibition for the growth of E. coli, S. aureus , Ps. aeruginosa. So how will be performed this material for the test of above test parameters ? Have any inactivator of Silver Sulphadiazine during microbial Count?

A1: Try using 4-aminobenzoic acid to neutralize silver sulphadiazine.

USP 2022

Anybody can confirm the dilutions in USP 2022 and 62 for following pathogens please.

S.aureus , E.coli and Salmonella . dilution 1/100 for all (USP 2022)

S.aureus , P. aeroginosa and E.coli dilution 1/100 and for Salmonella dilution 1/10

My second question is about the interpretation of Bile-Tolerant gram negative results:

In USP 2021 and USP 62 the interpretation is not the same.example: if we get 3 + results in USP 62 MPN is > 100 and in USP 62 is > 1000. Why it is different?

In USP 62 test for absence of BileTGN the volume of EE broth to use is not clear.

Is it 90 ml of EE broth? Is it valid to do Enterobacteria (Bile-tolerant gram negative) to detect coliforms & Aerobacter? Any reference& method for coliforms in USP?

FLUID THIOGLYCOLATE MEDIUM (FTM) in media fill

Can we use FLUID THIOGLYCOLATE MEDIUM (FTM) in media fill? If yes than please let me know in which condition we can use it.

A1: It is not recommended to use use ftm for mft as ftm is a media which has some agar and resozorin and also the characteristic ring formation requires heating of this media.

A2: You have to use Alternative thioglycollate medium (fluid thioglycollate medium without agar & resazurine indicator) for anaerobic media fill trials.

A3: First, I would only use FTM if my intention was to perform an anaerobic media fill. I would only perform an anaerobic media fill if:

- the process is performed in an anaerobic environment (like a nitrogen filled isolator) in which case and anaerobic media fill should be part of routine media fill program, or
- I have retrieved and anaerobic/microaerophilic contaminant from a sterility test positive (FTM media). (Note: It would be more difficult to retrieve an anaerobic/microaerophilic contaminant from an environmental sample unless you are anaerobically incubating these samples, the same is true of a TSB/SCDB media fill unless you have very little headspace in your media filled product units.)

If the media fill is intended to be anaerobic, I would want to make the conditions as anaerobic as possible by either minimizing the available headspace (and thus the available oxygen) or pulling a vacuum on the media filled units.

Secondly, if I were going to use FTM, I would use the alternative formula that does not contain agar if the plan is to filter the media as part of the media fill process.

You can a find additional information on anaerobic media fill in PDA TR #22, "Process Simulation Testing for Aseptically Filled Products" (1996).

A4: Given that a media fill is intended as a simulation of a product fill, you would want to at least consider doing an anaerobic media fill if your product and/or product filling conditions are likely to create an anaerobic or low-oxygen environment within your product, such as:

- if your product is very viscous (it will slow oxygen diffusion) or
- if you use a nitrogen or argon overlay to protect an oxygen-reactive product.

A5: The recommended media for APS as per Regulaotry is SCDM. Since it will support growth of all kind of viable microorganisms.

A6: We can use ftm in media fill activity whenever we found anaerobic bacteria in our environment.

A7: First give me the details of anaerobe you found in your clean room. What is room grade/class.? What kind of mfg. in your clean room? Please study the composition of FTM.
Refer media fill chapter in USP.

Lyophilizaer validation

Could you please briefly explain me regarding lyophilizer validation. What are the documents to be prepared? How to do the lyophilizer validation and how to prepare the documents for the same.

BioFilm Review Paper

A good review paper on Biofilms in

The biofilm matrix

www.nature.com/reviews/micro<http://www.nature.com/reviews/micro>

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SDA+ Antibiotic

I want to add an antibiotic to SDA to prevent the growth of bacteria,What do you recommend and what %? What are the references?

Any other specific method you recommend for this purpose?.Any supplier produces SDA+Antibiotic?

A1: Try adding 0.1 grams of Chloramphenicol/liter of SDA. By using antibiotics in SDA, there is the possibility of not detecting bacterial contamination in raw ingredients and finished products that you might not otherwise detect. For example, I had a problem at another company that I used to work for in which the presence of bacterial isolates in a test sample was only detected by using only SDA. These bacterial isolates could not be detected in a test sample by using TSA. It was determined that the contaminating bacterial organism had a lower optimum pH for growth (5.4 to 5.6) than what was the normal pH of TSA (7.3 +/- 0.2). If I lowered the pH of TSA to 5.5, the contaminating organism grew very well. I found out latter that it was a Gram-negative bacterial species that had adapted to grow in low pH conditions. My recommendation is be careful as to why you are using antibiotics in SDA.

A2: Better go with SDA with Chloramphenicol ready dehydrated powder or u can use Chloramphenicol separately to prepare SDA.

A3: To prevent the bacterial growth in SDA media, you need to add Chloromphenicol at the concentration of 50mg / L of media.

Reference : Indian Pharmacopoeia (2.2.9 Microbial contamination in nonsterile products)

The media is available in HIMEDIA Laboratories Pvt Ltd - INDIA (Code : M1067B).

A4: The product code is M1067 from Himedia make.

A5: We buy in SDA with Chloramphenicol manufactured by Heipha and supplied by Biotest UK. Settle Plates (30ml) and Contact Plates (diameter 55mm) both contain 50mg Chloramphenicol.

A6: You could use chloramphenicol (50mg/L); but try several % to find one that match your sample. Supplier sigma aldrich

A7: Alternately, one could plate on two types of TSA [one at pH adjusted to acidic] and other normal and take care of all different pH loving bacteria on TSA plates and have a bacteria-free isolations of only Fungi and Yeast on SDA by incorporating antibiotics.

A8: Could be worth investigating the use of 50/50% Chloramphenicol and Choltetracycline -this has in other uses shown better effect on bacterias and less harmful for the mould -important is also to try to avoid or minimize the use of ethanol while diluting the antibiotic powder if relevant.

Cleaning validation of equipment

I want to ask you about cleaning validation of equipment for nonsterile production. How do you calculate recovery and acceptance criteria? Are there any base guidelines where are described the microbiological aspect of cleaning validation. Thank you in advance.

Question Settle plate

During my environmental control for viable microorganisms, I used 1 petri dish per hour during a total time of 4hours. I obtained 4 results, is it ok to do the sum of the 4 obtained results or should it be the average?

A1: You should do a sum of the obtained results.

A2: You need to sum up the results of all 4 plates. I find this approach interesting because you will be able to detect and monitor more effectively the time periods wherein there is a maximum probability of contamination being generated in the area being monitored.

The trends of the individual results ought to provide sufficient information over a period of time.

A3: Either calculate the average and report result as CFU/plate/hour, or calculate the sum and report as CFU/plate/4 hours. Your SOP should reflect your decision, and your alert/action levels should also be based on whether you choose 1 or 4 hours.

<1111>

In the acceptance criteria for non-sterile product in chapter <1111> does not includ the search for Salmonella as specific organism, but in chapter <62> is described a methodology for the search of it. When we would have to search for Salmonella?

Culture suspension viability study

To perform GPT for media, I need 10-100cfu/0.1ml. culture suspension.Right now I am preparing suspension with 0.9%saline and I have to use suspension for 7 days which is stored at 2-8 degree C.But i am not getting recovery after 3 days. I did viability study using buffered sodium peptone chloride which showed increase in count from initial count.Then I did with phosphate buffer but no recovery from next day.

So please suggest me better method for culture suspension preparation and viability for 7 days.

A1: We do not perform GPT at nearly the frequency that you do, but for our purposes we only allow the buffered solutions to be used for a maximum of 24 hours. I would suggest that once you grow out your organism in broth, put it at 2-8°C to slow growth. This solution you can use for a week or so. Perform your dilutions up to 10-7 in duplicate to figure out what dilution will get you 10-100 CFU/0.1mL. Then throughout the week you can make the dilutions as needed.

A2: You need to validate the expiration date of your suspension before you start using it. Low nutrient medium usually does not support organisms during storage especially if you want to store low concentration of organisms.

The best way to store low counts is to freeze the culture in small vials and then to defrost prior to use.

antimicrobial activity of bar soaps

Pls has anybody done some work on antimicrobial activity of bar soaps. I am doing a work on it but have some worries.I use agar diffusion method and concentration of soap were 0.1g/ml - 0.0001g/ml. But no inhibition was found. Please do I need to increase the concentration of the soaps or is there any other method i can use. thanks.

A1: I have two questions for you. Are you screening the effectiveness of different soap bar formulations? Does you soap bar contain an antimicrobial agent such as Triclosan of TCC? If a soap product does not contain an antimicrobial agent, you are just removing bacteria from the skin and there has been alot of disagreement as to whether antibacterial containing soap products are more effective than plain soap products which I'm not going to get into at this time. That is a whole different discussion. There is one method that can be used to demonstrate the antibacterial effectiveness of soap products. It is ASTM Method called Standard Test Method for Evaluation of the Effectiveness of Health Care Personnel or Consumer Handwash Formulations E1174-00. It is a microbial clinical in-use study.

A2: Soap solution will not diffuse into the medium . you will have to make different dilution of it and dip round discs of about 8 mm in each dilution and the put it on agar media previously containing pathogen with solidified media. and also try lower dilutions like 10 gm/1ml and its serial dilutions.

A3: If your soaps contain antimicrobial substance take care interpreting results form diffusion assay.

1 diffusion will not differentiate between biocide and biostatic activity

2 Zones of inhibition may reflect rate of diffusion of the compound not its activity

3 Compounds in your growth medium may inhibit some antimicrobial substances

A4: "If" your soaps contain antimicrobial substances take care interpreting results from diffusion assay.

1. diffusion will not differentiate between biocidal and biostatic activity

2. Zones of inhibition may reflect rate of diffusion of the compound not its
activity

3. Compounds in your growth medium may inhibit some antimicrobial substances

CEN standards such as 1499 are designed to evaluate bactericidal handsoap quantitively

A5: I'm a little surprised, because the detergents in classic soaps like curd soap, have no or less antimicrobial activity. The reduction of microorganisms results normally from the mechanical effects of the hand washing procedure. In case of a soap including antimicrobial detergents like Triclosan or others.

Maybe the test procedures of EN 1499 and EN 13727 will be helpful for you. Furthermore, I remember that there was a comparison of different soaps/washing lotions and hand disinfectants published by Kampf and Kramer (Comprehensive evaluation of the most important agents for hand hygiene, 2004.

A6: I would try to determine which of the ingredients in the antiseptic soap bar has antimicrobial activity. I would suggest that the first step is to test them against organisms that are found on the skin by performing Minimum Inhibitory Concentration and Minimum Lethal Concentration testing if they are not a known active such as Triclosan, TCC or Benzalkonium chloride. In the US, antiseptic and antibacterial soaps are regulated as drug products. Most people are using the ASTM E1174-00 method to demonstrate antibacterial activity. I know that there are several EN test methods that people use in Europe for evaluating antibacterial soaps, but I'm not familiar with them. The problem that you are going to encounter

in these types of studies is the mechanical removal of organisms by the soap itself from the skin and it has nothing to do with antimicrobial substances unless you perform some type of comparison study in which the antiseptic soap has a greater reduction than the soap without the actives. However, you will run into alot of problems in trying to formulate a soap with and without an antimicrobial active. You have to remember the amount of antibacterial activity is also dependent upon how the formulation was put together because antibacterial soaps are not found to be equal in effectiveness due to this reason.

A7: Could you also do a 'ring of inhibition' or Kirby Bauer type of test using the various pathogens you are interested in? This test is alot quicker than the MIC test.

A8: If you want to screen product formulations for antimicrobial activity, a zone of inhibition of a Kirby Bauer Test (Disk Diffusion) can be used. However, they are crude methods. Besides whatever actives that are being used to claim antimicrobial activity in a bar soap, you have to remember that certain anionic, nonionic and cationic surfactants that may be present in a soap bar can also contribute somewhat to the antimicrobial activity of the bar. See Chapter 4 - The Roles of Surfactants in Self-Preserving Cosmetic Formulations in Preservative-Free and Self-Preserving Cosmetics and Drugs, Principles and Practice by Don Orth and Jon Kabara.

Furthermore, the test data generated by these 2 methods (e.g. zone of inhibition or Kirby Bauer) cannot be used to make antimicrobial claims for a product because antibacterial soap products are covered in the USA by a FDA Tentative Final Monograph. Antibacterial soaps in the US are considered to be OTC drug products. As part of this tentative final monograph, ASTM E1174 has to be used to generate the necessary supporting data to make antibacterial claims by conducting a clinical in-use study in which the hands of individuals are purposely contaminated with a microorganism and a recovery study is conducted after use of a soap product.

Pencillin Assay

Our sister co. is having problems with Microbial Assay of Penicillin. The product contains two types of Penicillin derivatives. During sample dilution, a lot of foam is created which is probably the reason for low assay values. Has anyone experienced this problem and if yes, how can it be solved?

ASTM standards for efficacy testing of disinfectants and modification in terms of organisms

If anybody is using the following standards to generate efficacy data to show antiviral activity of a disinfectant, I would like to have some advise :

1. ASTM E 2197-02 method described use of Canine Parvovirus. Can you use this method to show the activity against Vaccinia Virus or ROTA virus or even Aspergillus?

2. ASTM E 1053-97 test method is for vaccinia and Rota- human strain. Can you use this method for canine parvovirus?

Do you need to validate these methods if you change the organisms described in these standard test methods and how can you validate these tests?

Sterility Test Validation of Lubricating Jelly

I need some guidance regarding sterility test validation of Lubricating jelly (sterilized by Irradiation).

As the product is not filterable by nature, I have to perform the validation by Direct Inoculation Method.

But the product contains some preservative (paraben) so I'm looking for the guidance, what are the neutralizers with concentration should I consider during validation?

How should I use that neutralizer in the Direct Inoculation Method? - Should I have to incorporate the neutralizer in media (SCDM & FTM) before sterilization?

A1: Neutralizer - Lecithin.

Yes, first you have to validate the concentration (1%, 2% up to neutralization effect).

After validation you can incorporate the validated lecithin concentration in SCDM & FTM and sterilize.

A2: Perhaps a more fundamental question is why a multi-use preserved vaginal or rectal jelly a sterile product?

Sterilisation of herbal extracts

Can olive oil extracts of Calendula offcinalis, Symphytum officinalis and Hypericum (or any other medicinal herbal extract) be sterilised in any way, without affecting their anti-inflammatory, antibacterial, antiviral activity?
Can you provide me with some literature on this topic?

Surveying the net I found out that these extracts are used in products for treating open wounds - and I know that some authorities (for example European Pharmacopoeia) insist on the sterility for such products. How can sterility be achieved in a product that contains herbal raw materials?

Does anyone have similar products? Do you produce them as sterile, or do you only set more stringent requirements than usual?

A1: You can check with STERIGENICS.

A2: I'm not sure how viscous these extracts are, but you could possibly consider sterile filtration. I would imagine that any sort of heating would damage the product, and EtOH or VHP would probably be difficult sterilization methods to use here.

A3: Depends if you make a ttherapeutic claim in which case You need to be able to identify the active ingredient(s), provide proof they (it) work on the organisms cited, quantify it(them), ensure you deliver a consistent therapeutic dose, few clinical trials etc...That should take ten years. There might be something new in terms of sterilising by then.

If you want to put something on the market with no therapeutic claim except its sterile The options are irradiation, heat, filtration. But a natural product also has to be carefully handled from harvest, through extraction and storage. Poor handling might allow microbial growth with the inevitable endotoxin even in a sterile product. For me its heat or irradiation in either case you will want to show no loss of efficacy after the event.

You could investigate critical C02 extraction which in itself is quite a harsh process and may reduce microbial contamination in which case you may be able to use use preservatives.

Or go the Honey route, sell it as food but leak in the press it has remarkable heaing properties.

regarding the minimum quantity of API for MLT and sterility

I am having a doubt regarding the minimum sample quantity of API to be used for MLT (one time analysis and validation) and sterility(one time analysis and validation).If we are having a market sample to be analysed for the same and the material is very costly what should be the minimum quantity for both of these tests.

Pl. also guide me if any references are available.

BET test of finished products (filled vials or ampoules)

While performing the BET test of finished products (filled vials or ampoules), testing of single vial or ampoule from a batch or lot is acceptable or we need to test the pooled sample. If pooled sample is preferable, how many number of articles shall be pooled from a lot or batch.

Any reference pl mention.

A1: I am sure you will get plenty of answers to your question.

Once your product is validated, I would suggest that 3 samples of individual vials be tested. One each from beginning, middle and end of the production run, to show reproducibility across the batch.

If you do consider pooling then consider also the possibility that a good sample could mask a bad sample. My advice is that pooling is not preferable.

If you decide to go down the road of pooling samples, a general rule is that you should divide the Endotoxin Limit for the product by the number of samples pooled.

So, if your limit is 2.4 EU / ml for the product and you have pooled 3 samples you should test at a limit of 0.8EU/ml.

A2: There is an explanation on the sampling technique in the FDA Guideline published in 1987, GUIDELINE ON VALIDATION OF THE LIMULUS AMEBOCYTE LYSATE TEST AS AN END-PRODUCT ENDOTOXIN TEST FOR HUMAN AND ANIMAL PARENTERAL DRUGS, BIOLOGICAL PRODUCTS, AND MEDICAL DEVICES.

It says "The sampling technique selected and the number of units to be tested should be based on the manufacturing procedures and the batch size. A minimum of three units, representing the beginning, middle, and end, should be tested from a lot. These units can be run individually or pooled. If the units are pooled and any endotoxin is detected, repeat testing can be performed. The LAL test may be repeated no more than twice. The first repeat consists of twice the initial number of replicates of the sample in question to examine the possibility that extrinsic contamination occurred in the initial assay procedure. On pooled samples, if any endotoxin is detected in the first repeat, proceed to second repeat. The second repeat consists of an additional 10 units tested individually. None of the 10 units tested in the second repeat may contain endotoxin in excess of the limit concentration for the drug product."

Therefore, three samples from the beginning, middle, and end of the production are recommended to be tested.
Pooling can be used, but the endotoxin limit for the pooled sample should be lowered according to the numbers of the units pooled. For example, the endotoxin limit should be 1/3 of the original if you pool 3 units of the sample.

A3: GUIDELINE OF THE LAL by US FDA released in Dec 1987 mentioned

"A minimum of three units , representing the beginning, middle and end, should be tested from a lot. these units can be run individually or pooled."

Minimum no. of plates.

I want to know that is there any formula for the minimum no. of settle plate (passive air sampling) that has to be expose related with the size of the room during area qualification of aseptic area.

Mine aseptic room size is 15 meter (length) x 12 meter.(width).

A1: There is not a minimum number of plates according to the size of the room.
You need establish the sample points according the design, the air flow, dead zones, the critical zone, the classification of the area, historical results, etc.
Your sample plan has to assure that the microbiological status of the room is adequate to perform the work.

A2: Microbes do not know the mathematics. Neither we can teach them. No formula can be a all inclusive criteria. You may decide the number of plates based on the below. Critical areas that if contaminated will affect the product quality Non-critical sites that may not affect the product quality but may indicate the worst load in the area Sites where the maximum air sweeps back in the rooms (you may check the air flow patterns in the area). Sites where there is high operator movement (high traffic).

A3: The number and location of settling plates depends on the process occurring in the cleanroom. There is no formula used to calculate a minimum number of locations.

Expose settling plates near critical activities but do not negatively affect the environment. You should write a rationale for site selection that documents the reasoning and risk.

A4: The number and location of samples should be based on a risk analysis, taking into account, activities / interventions, air returns etc in the area

It would be expected that the number of locations are not less than the number of locations required for qualification of the area in accordance with ISO 14644.

A5: I would be interested in the type and design of your sample site selection risk assessment. We are about to started performing a sample site risk assessment at our facility and I would like to understand what industry is doing. We are a non-sterile drug substance facility.

A6: Can you suggest me, the risk analysis for viable as well as non viable particle count is carried out in which stage of qualification? (at rest or in operation).

A7: in that case formula used under route A where A is area of room.

E. coli in a raw material

In a recent test for specified m.o. (in this case, E. coli) in a raw material, I've found some lac - colonies in MacConkey Agar, and, in order to know what was de mo (of course, I supposed that was not E. coli), after a biochemical kit I realized IT WAS AN E. coli.

Then, I done some test in order to verify the result:
. Indol: +
. growth on Levine Eosyn Methylene Blue: not typical (pink colonies with no metallic sheen)

After that, I've sent the colony to an external laboratory in order to confirm the identification and they found an E. coli (with this rare behavior too). Consulting to one recognized colleague of my country, she told me that it was an inactive E. coli.

Then, I'd like to know if you have faced out with this strain and if you know some supporting bibliography about it.

A1: What is your primary purpose, ID or confirmation of biochemical repertoire?

If ID, then consider using sequencing. It appears that both you and your reference lab are relying on phenotypic methods for IDs. Sequencing or RFLP for subtyping will give you a more reproducible answer.

A2: Aame strain of E.coli can't grow on EMB agar as colour of metallic sheen. So, you can isolate this colony and than identify with API 20E. I suggest it.

A3: I have isolated lactose negative E coli. i love the real world. I pasted an article below to help you start your search for information.

[name redacted]

Identifying strains of E. coli
Medical Laboratory Observer, April, 1998 by David L. Sewell

A4: We too have picked up Lactose fermentation negative E. coli, but haven't been told it was an "inactive" strain...not heard that before! I was under the impression that the inability of E. coli to ferment lactose was associated with an inactive Lac operon, which may be the point of the "inactive" reference.

Somewhat interestingly, and I'll stand corrected on this, the really pathogenic strains don't do the MUG reaction which I believe is also indicative of this operon being inactive, or mutated for something else (perhaps resulting in extreme pathogenicity?). Is it possible that you have isolated OH157 or similar? If so, I would be very careful indeed of handling Lac negative E. colis... more on this idea from anyone would be very welcome...I may be jumping at shadows.

We did still treat it as indicative of faecal contamination, with subsequent assumptions of the presence of other nasties, which is the main point of the test.

A5: Have you re-tested the raw material, you might have a situation where the bag was contaminated and it has contaminated your sample.

Eliminate this situation first by re-sampling as often, as stevedores on docks and loading bays, once the load is full, they often walk on top of the bags thus spreading all kinds of mo's with their shoes.

I have found this situation many times before, ensure that the site where the sample is taken if a paper bag is swabbed properly before sampling, many times samplers use sampling thieves contaminating the same itself!

If none of the above applies, well then by all means go ahead and have it checked.

A6: Think you'd need to prove sampling error before relying on a retest.

A7: May this is not a correct solution.
As each and every container (bag) can be checked for the absence of the organism and the process is tedious. It is the duty of the supplier to ensure proper packing to avoid all the happenings given below.

A8: i have a similar kind of doubt, that is, CAN Bordetella pertussis grow in FTM or SCDM? Could you clarify me.

petrifilm use

I just wanted to see if anyone in the dietary supplement industry has validated the use of petrifilm for Micro testing. If so, how do you feel it compares to the USP methods for dietary supplement? I work for a liquid nutraceutical company and they have been tossing around the idea of using petrifilm for Micro testing. I know that petrifilm is widely used and accepted in the food industry (Dairy-for example), but not so much

so in pharmaceuticals and dietary supplements. Any feedback or thoughts on this matter will be greatly appreciated. Has anyone discovered any disadvantages to petrifilm?

A1: Hi. I would suggest that you call 3M and ask for a representative to come out and help you decide if you want to use the petrifilm system. Many years ago I used the Total Aerobic Plate Count media and the Coliform testing plates in the dairy industry.
The dairy industry is highly regulated so a USDA person would stop by periodically and take their own samples for testing.
So our samples were information only. I liked the system and representative was very helpful. One good point is only a small incubator is required because the test cards are so thin.

In the pharmaceutical industry the idea of using petrifilm has never been met with enthusiasm.

Depending on your testing you may need to qualify using the petrifilm system against standard media in petriplates.
Most likely the criteria would be the petrifilm results must be within 50-200% of the standard media.
One USP chapter states, I believe, that duplicate plates (%RSD) must be within 30% of each other, but I am not sure if people follow that.

Growth promotion testing on each lot of petrifilm would be a good idea.

A2: I'm sure that there will be many more responses to your question, but to start off, the Petri Film test for E. coli and coliforms is not able to be validated as equivalent to harmonized USP due to it not being able to include the full group of BTGNs. Coliforms are an acceptable test in USA for foods (AOAC approved), but not for therapeutics. I also seem to recall that the colony colour based identification of E. coli might not pick up all those that USP method is capable of, but I'd bow to better informed comment on that one.
On a personal note I hate the things; fiddly, potentially messy, unconvincing...apologies for emotive rather than scientific!

A3: Have found petri films useful & handy for certain purposes but there are caveats. For the APC (TAMC) it often depends on the scope of testing intended. Was involved in one situation where there was a lack of coherency between In Process (Petri Film) results and Finished Product (Standard Pour Plate) due to the fact that the shallow depth of agar on the Petri film format did not support recovery of microaerophiles such as lactobacilli while the standard pour plate allowed better recovery and so the counts were sometimes significantly greater on the finished product than were predicted by the In-Process counts - rendering the IP testing worthless.

Worth bearing in mind that if more than one method is employed, coherency between the different methods be established in actual trial conditions rather than theoretical qualification tests based on testing with a limited number of prescribed Growth Promotion strains that would indicate equivalency between methods.

Just another example of where Microbiological Profiling of the background bioburden of raw materials and in process products is not an option but a pre-requisite for good microbiological practice.

A4: We are a food and dietary supplement manufacture. We tried petrifilm for our APC and Y&M counts a long time ago. Although we could show the films were OK for some of our products, others reacted to the media and did not allow the films to solidify. We currently use them for Environmental monitoring only. The only advice I can give is to try them out. You will have to validate the method results with your other testing. With the coliform film, we have been successful at recovering the organisms off the film for further identification and confirmation. Testing for E.coli and Salmonella would still require your regular testing since we use enrichment methods. Years ago I would have said the cost of petri film was prohibitive since media is so cheap, but with the requirements to growth promote each batch of media prepared, maybe it would be more cost effective to use petrifilm?

Switching LAL Methods

We are interested in changing our current LAL method which we use for our release testing. Does anyone know what the regulatory impact would be regarding this issue?

A1: You would have to re-validate all the products you intended to test with the new method, and subsequently update all of the DMFs to include the new method.

A2: As a general rule when changing methods you will need to perform a three batch revalidation of your product.

If you keep to the same method, but change suppliers, this is a single batch revalidation.

There is also a third revalidation requirement which is when there has been a significant change in production of a product. Now define 'significant'.

The above can be found in the FDA Guidelines.

A3: Revalidation for each product will be required. Positive approval of the change will be required of the regulatory agency.

A4: The impact will depend upon the level of detail that was included in the drug filing. Your own regulatory department will need to make the decision as to whether the change would be a CBE30 or annual reportable change.

You should investigate what was included in the drug filing.

Media Fills and Fill Operators Qualifications

I have been reading the FDA guidance Document on Aseptic Processing and the EU Orange guide. Process simulations using media should be done twice per year per shift and per process. The FDA guidance document states that personnel should have annual participation in media fills.

Does anyone know if there is a criteria that fill operators must qualify twice per year? Is twice per year industry standard? I can only find references that say annual.

A1: You might find additional guidance in ISO 13408 Part 1.

A2: Annual is the regulatory requirement and pretty much the industry standard. You can do it twice a year if you feel your process needs it that often.

A3: This is really two separate issues.

Every operator should participate in a process simulation media fill annually. This should be a requirement of all personnel who work in the clean room including QA, EM technicians, mechanics, etc.

For each process, each manufacturing shift should perform media fill every six months.

So if you run one operation over two shifts, each shift would be required to participate in a simulation every six months.

A4: Agree with [name redacted]. I am posing few more questions related to media fill.

Does the industry wait for the media fill results in case of a re-qualification or just perform the media fill and go ahead with the routine production? I am personally of opinion that we don't need to wait for the

results. But wondering what is the industry standard / regulatory prospective on this? In case of introduction of new pack, the new pack is found not adding worst case to your already validated line based on the risk assessment, Is it still required to take 3 runs? How the industry is interpreting this kind of pack?

A5: Regarding with the criteria of the operations and operator for the Aseptic Processing I would like to confirm whether a technician infected with Hepatitis B virus and still a career is qualified or conform with the guidance of the GMP or GLP. Please do give the suggestions.

A6: I think based on Risk assessment and historical media fill data we can go ahead for commercial batches rather than waiting for results.

I have one more question regarding use of FTM instead of SCDM for the process which use Nitrogen gas for used in product filling of PFS? can any one cite me guidelines for above said case and how oftely we have to simulate like annually or twice an year?

A7: Operators are qualified once per year. This is what is practical with shifts, absenteeism, business needs etc.

A8: [name redacted] pretty much nailed it, the expectation is two media fills per year with individual components of the process challenge (e.g. line speed, container size, etc) having separately defined frequency requirements. Personnel qualified to work in the clean room should each participate in a media fill at least once a year. One bit of insight I'll share is regarding personnel vs gowning qualification. I've had European inspectors indicate that media fill participation is not a substitute for a stand qualification program for clean room gowning. I've always had both so it wasn't an issue but thought I'd throw it out there.

Regarding waiting for results, I not only require passing results from the media inspection but also from post incubation nutritional adequacy testing of the media. I've never been asked directly about it but I'm not sure how I would justify updating personnel/process qualification due dates without verifying that the media results since a failure would call all of that into question.

A9: Regarding an operator infected with Hepatitis b. Hepatitis B is 100 times more contagious than HIV, the virus that causes AIDS. In addition, the hepatitis B virus can survive outside the body for at least seven days and still cause infection. So in my opinion this operator should not be involved in aseptic processing. That is the same reason we don't allow operators with colds or open cuts to work in an aseptic processing area.

A10: As far as I know, the only qualification for a fill operator is that they are able to pass personnel monitoring (gown and finger tip plating). If an operator sheds microorganisms excessively and they fail gown platings they may be disqualified. If their gown plates have pathogens associated with the organisms (even in low numbers) they may be reassigned until that issue is resolved. People who take certain acne medicine can become shedders and have Staph. aureus on their plates. In this case they must be reassigned until the skin problem is resolved.

Hepatitis B is not easily transmitted to others. If you have hepatitis B you should not share a toothbrush with others or better yet get your sex partner vaccinated.

I think it may be against the law to ask peoples status on diseases, such as HIV, so the only information you would have are the gown plates. If those are good, low or zero with no pathogens, then no one should be asking questions. If yeast keeps popping up there
May be questions. Personnel with an infectious bacterial disease or is in viral outbreak period (herpes) should not be aseptic operators.
The question is very controversial.

Continuous monitoring in pharmaceutical industry

Please advice, My company will be installing a new continuous environmental monitoring. Since that is new to us, I am really not sure as to where the detector probes should be placed in our aseptic filling area and the filling line itself.Does anyone know of a journal that I can get the referencing on.

A1: I refer you to "Best Practice for Particle Monitoring in Pharmaceutical Facilities (Technical Monograph)" as a good getting started guide.

A2: This is really two separate issues. Every operator should participate in a process simulation media fill annually. This should be a requirement of all personnel who work in the clean room including QA, EM technicians, mechanics, etc. For each process, each manufacturing shift should perform media fill every six months. So if you run one operation over two shifts, each shift would be required to participate in a simulation every six months.

AET

I have a question about the antimicrobial effectiveness test.
Since the initial inoculum required is 10E8 cfu/ml so after inoculating 0.1-0.2 ml into 20ml of media one gets 10E5-10E6 cfu/ml; how do I perform the confirmation for the inoculum size? Do I dilute the stock in order to get a final concentration of aprox 100 cfu/ml. I'll appreciate any insight.

A1: Try inoculation of an equivalent volume of saline solution in place of the test article and undertaking serial dilutions to determine the count?

A2: As far as I recall the initial inoculum during a preservative (AET) test is 10E5-6 CFU/mL of the product. You can establish the initial inoculum by inoculating the same volume of saline (can also add 0.05% Tween 80 as surfactant) - at the same time as you are inoculating your products. That is if you volume of product per organism is 10 mLs, then set up another saline blank with 10 mLs in it, and inoculate it at the same time as the product.

You then need to dilute both the product at the time of testing, or the saline on the day of inoculation (ASAP) in some sort of diluent - with a series of 10 fold dilutions. When you get to the 10E-3 and 10E-4, place 0.1 mL onto an agar plate (which is another 1:10 dilution). The 10E-3 dilution plate should have between 10 and 100 colonies if you have prepared your initial inoculum correctly, and the initial inoculum should be confirmed to be 1-10E6 CFU/mL. We used to plate the 10E4 dilution just in case the inoculum ran high, but generally the count per plate is too low to be statistically signifficant.

A3: You are right about the final inoculum size (10E5-6 cfu/ml), but that is after I inoculate 0.1-0.2 ml of microorganism suspension / 20ml of media+product, my question is if I am allowed to dilute the initial suspension of 10E8 cfu/ml to 10E3 cfu/ml using 10 fold dilution in saline solution in order to plate it and have an accurate count ?

A4: In our company we dilute the stock to obtain 10-100 CFU/ml to be inoculated and incubated for 5 days. The result of plating then multiplied w/ diluent factor.

A5: If I use TSB broth with Lecithin(5g/l) and polisorbate(40ml) for the recovery of microorganism after the incubation period, do I have to validate such neutralizers per chapter <1227> ?

A6: You only need to show the non toxicity and the effectiveness of the neutralizers used.

A7: The answer is simple - yes. Each combination of neutralizing broth and preservative needs to be validated. In addition, a lot of people perform a quick validation test along with each test they do. At the very least, you will need to perform a large validation in triplicate for any registration.

A8: Do I also have to validate per chapter<1227> the dilution I'm planning to plate for the confirmation of the inoculum size so the estimated countable CFUs fall between 25-250 cfu for bacteria and 8-80 cfu for Aspergillus?

Component Testing (Caps, bottles)

Is anyone using phosphate buffered saline (PBS) minus the tween for recovery of potential microorganisms through membrane filtration (0.45 um).

We are currently rehydrating PBS tablets that have 0.05 % Tween 20. It seems to slow down the filtration rate.

Harmonization of IP

I want to know that whether Indian Pharmacopoeia (Microbial limit test) is also harmonized with USP <61> & <62> or not.

A1: Still IP - 2010 not harmonized.

Particle count vs viable count

As per EU annexure-I and ISO-14644 there is two limits for particle count at rest and dynamic condition. For example In “B” grade 0.5μ limit is 3520 for rest, 352000 for dynamic condition. Logically while dynamic condition there is a possibility of particle generation and turbulence of air. For that reason the particle count limit is higher when dynamic condition but why the same condition is not applicable for viable count (increasing count). There is single limit for viable count. Plz clarify me.

A1: Would guess that the difference is due to that the particle count is used to measure air cleanliness and as a "real-time" indicator for viable contamination. Actually it is the viable particles which is of greatest concern but limits are only recommended specs as technology is not available today to measure and ID viable particles real time -and total particle count is used as surrogate. -Some say non-viable count but it is actually the total as the particle counter doesn't differentiate.

Particle counts will be highly influenced by the presence of humans but it is critical that it doesn't give rise in the viable counts -as this is a cleanroom!

A2: Actually there are now commercially available optical spectroscopy technologies that provide simultaneous total particle counts and viable counts in real-time. I have written a number of papers and given presentations on one of these technologies, the BioVigilant IMD-A. A list of these publications may be found on the Reference Page at http://rapidmicromethods.com.

A3: The conditions for 'at rest' are specifically in annex 1 stated as "with no personnel present". As personnel are the main source of viable particulates in air, no 'at rest' limits for viables are provided.

Suitability in presence of product

For a while we have been trying to perform a Suitability test for Tinidazol tablets, with no recovery results, we tried TAT broth, D/E broth, and both diluted up to 1/400, no luck.
I was wondering if we could use any other neutralizer to inhibit the tinidazol antibacterial activity. any other idea very much appreciated.

A1: Try the suitability and recovery by addition of polysorbate 80,
Follow the membrane filtration for recovery with suitable rinsing fluid and validate the no of rinses
Refer PDA Tech report 33

temperature specifications inside of aseptic filling suites

I was wondering if someone could help me find documentation on temperature specifications in regards to aseptic filling rooms.

Settle plates

A quick (and hopefully easy) question: does anyone expose settle plates for any shorter than 1 hour during routine unmanned microbiological monitoring. The limits in EU GMP are set on a 4 hour exposure and most people I know expose only for 1 hour...for a myriad of reasons e.g. plates drying out , insufficient time , production requiring the area etc.

A1: Settle plates to be exposed for NLT 4hrs as per guideline. You need to use 32mL media per plate instead of 18mL media and demonstrate that it passed growth promotion and recovery.

A2: Our Company in Indonesia doing exposure plate for 30 minutes. This is based on Indonesian Guideline on Pharmaceutical by our local FDA.

environmental isolates

I need some help regarding the isolation and identification fo Environmental Isolates which are found in the manufacturing facility and sterility testing area of microbiology lab.
For identification of bacterial up to genus level presently we are doing grams staining followed by the inoculation of the culture in the kits of MINIAPI bacterial identification system for different organisms depending on the staining characteristics.

Here my question is is it sufficient to identify the organism or we must perform the biochemical tests for the isolated bacteria.

I need help in this regard and if we have to perform the biochemical tests what are all the tests that are required for the identification of bacteria that are found in the environment that we have to perform.please give the detailed information which will help me in implementing the same immediately.

A1: Correct me if I am wrong as I have not worked with the miniAPI, but the biochemical testing is done automatically within the machine using the testing strips. This is how the identification process works with the API and comparable competitor products. The miniAPI system even has the capability to test sensitivities (another source of ID). It seems to me that you are already set, however if you wish to verify your results you can purchase selective medias and/or a secondary ID system or even a PCR machine. It really depends on how far you would like to take this process.

For environmentals we ID and do a sensitivity, that's where we draw the line. Performing all the biochemical testing manually (and individually) would require a lot more time and resources, something our lab cannot spare for such testing.

A2: For our facility we did and study for 2 year to identify the environmental flora. the identification was carried out up to the API (with G. Staining, catalase or oxidase test) (some up to species, some up to genus), and that was sufficient.

A3: For identification of isolates recovered from a Manufacturing facility, different automated Microbial Identification Systems can be used such as MIS from MIDI, Riboprinter, MicroSeq, Vitek. In order to verify and conclude with confidence other diagnostic tests and biochemical tests should be performed. Also note that the systems used for identification of microorganisms have to be validated prior to use.

EU annex 1 settle plate monitoring

For those of you that perform settle plate EM for all cleanroom classes (e.g. ISO 5, 6, 7, 8) according to annex 1, do you perform this in all classified rooms including material and transfer airlocks, or do you only focus settle plate monitoring in actual areas where the production activities occur?

A1: At a minimum you want to monitor the areas of production (critical areas). All other areas can also be done for additional information. The preferred method for EM is to use an active air sampler in all areas within the cleanrooms rather than settle plates which are usually for reference only.

A2: in our facility in Indonesia, we performed settle plate in class A (<1CFU) and class B (<5CFU) every batch production. Class B include airlock, change room, unloading area, trolley storage and corridor .

Even these room not directly contact to product, the result will reflecting personal activity inside the room.

Definition of Gram Positive organism

How many people would agree with this definition of Gram Positive organism.

Gram Positive organism: An organism that retains the Gram Stain.

A1: I would modify to define it as : Organism that retains the crystal violet stain in a Gram Stain is a G+ organism. There is no stain as Gram Stain!

A2: A better description would be an organism that retains Grams Crystal Violet stain after decolorization. The term "Gram Stain" could apply to all the stain reagents in a Gram stain procedure (i.e. Grams Crystal Violet, Safranin or Basic Fuchsin).

A3: I have to disagree because the gram you want retained is the crystal violet-iodine complex. Also do you intend to define GVs gram variable cells?

A4: For a illiterate (who don't have the knowledge of microbiology) person this is the perfect definition. But not for a qualified person and microbiology point of view.

A5: I'd defer to the test - an organism that retains Gram stain after decolorization by the method Bacteriological Analytical Manual R32 - Gram Stain.

A6: The stain name is just from the originator Hans C. Gram and as you know, Gram-positive bacteria have a thick mesh-like cell wall made of peptidoglycan (50-90% of cell wall), which stains purple while Gram-negative bacteria have a thinner layer (10% of cell wall), which stains pink. Thus the positive is for the crystal violet first applied and then trapped by the iodine and 'if purple it is positive'. The decoloration and counter stain is to be able to 'see' that the cells are still there and did were not fix'd with the CV and are pink. So if an organism by use of this method is purple, it is gram positive.. agree with definition.

A7: Are we looking at this right side up? The explanation of retaining stain/mordant is technically correct for explaining the stain, but does it adequately explain what is classified as a Gram positive cell? Fundamentally, what really distinguishes a Gram positive cell from other cells is cell wall and cell membrane structure. Lots of things can retain crystal violet (cotton, for example), but that doesn't make them Gram positive.

A8: I threw that question out there because that definition was given in the newest publication from a well respected organization. They also messed up a few other definitions. I agree the definition is poor. The need for qualified persons on these committees is pretty evident.

swab recovery studies

I'm trying to perform a swab recovery study for EM on filling nozzles in a Grade A area. I'm sure you will finds loads of information about swab recovery studies for qualification of disinfection programs, but for EM method validation, there seems to be very little, can anyone point me in the right direction?

A1: Why would you have to do recovery studies on swabs from fill nozzles? For fill rooms things have to be sterile so you swab a surface and put the swab in broth. If the broth turns turbid there is a problem. You are not enumerating the number of bacteria because even one would be a problem.

They sell systems that the sterile swabs with the sterile media in a ampoule. You swab, put the swab back in the container, break the ampoule on the media to flood the swab with media, and then you incubate for at least 14 days.

A2: There is an article published in the March 2010 PDA Journal of Pharmaceutical Science and Technology that you may want to read. It's titled, "Evaluation of the MicroWorks, Inc. Swab Sampling System for Use in Performing Quantitative Swab Sampling" by Sandy Rubio, Dawn McIver, Natalie Behm, et al. This six-part study was conducted to qualify the swab kit for use in sampling cleanroom surfaces for bioburden.

A3: Dangerous area to get into, swab recoveries are often poor. Once you know that, then what?

Anyway, some references:

Favero, M., McDade, J., Robertsen, J., Hoffman, R. and Edwards, R. (1968): ‘Microbiological Sampling of Surfaces’, Journal of Applied Bacteriology, 31: 336-343
Niskanen, A. and Pohja, M. (1977): ‘Comparative studies on the sampling and investigation of microbial contamination of surfaces by the contact plates and swab methods’, Journal of Applied Bacteriology, 42, pp53-63

Poletti, L., Pasquarella, C., Pitzurra, M. and Savino, A. (1999): ‘Comparative efficiency of nitrocellulose membranes versus RODAC plates in microbial sampling of surfaces’, Journal of Hospital Infection, 41: 195-201

Whyte, W., Carson, W. and Hambraeus, A. (1989): ‘Methods for calculating the efficiency of bacterial surface sampling techniques’, Journal of Hospital Infection, 13, pp33-41

A4: You may find the following reference useful for further information on Nylon-flocked swabs that are now available and effective for fully quantitative sampling of surfaces during environmental monitoring:

"Qualification of High-Recovery, Flocked Swabs as Compared to Traditional Rayon Swabs for Microbiological Monitoring of Surfaces"
Gilberto Dalmaso, Manuela Bini, Roberto Paroni, Michela Ferrari
PDA J Pharmaceutical Science and Technology. 2008 May-June Vol. 62, No.3, pgs 191-199

endotoxin in sterile API

Regarding sterile API, is there any mandatory requirement to include bacterial endotoxin test when it is not mentioned in the monograph?

A1: Check the general notices to see if there is anything there, and of course whether there are controls on the manufactured product that will require controls on the API in order to meet specifications.

bacteria in 70% IPA (spores)

Can anyone in here provide me any references or perhaps personal experience of growth of bacterial endospores in 70% IPA that is not subsequently sterile-filtered? In what quantities have you seen them, say, in a reusable spray bottle filled with IPA from a larger stock of unfiltered IPA?

A1: We would find test non sterile alcohol by filtration using teflon membranes and get 1 to 50 CFU in a 500 mL sample on a regular basis. The growth came from Bacillus spores in the alcohol.

A2: Spores will not grow in 70% IPA. But they are also not killed by either IPA or ethyl alcohol. Liquid spore suspensions are stored and shipped in 70%-95% ethanol and spores can be aspirated into a spray bottle (via the air drawn in to equalize the pressure) and will survive well only to be subsequently sprayed onto surfaces. All alcohol used for disinfection should be filter sterilized. Spray bottles or aerosol cans of alcohol used in aseptic areas should be sterile inside and out.

A3: I have also come across Bacillus spores in IPA which has not been filtered. Using the same bottle twice, and presumably decanting, would increase the risk.

A4: What is the frequency at which you perform this exercise? Is the 70% IPA prepared in-house or bought prepared from a supplier? If prepared in- house, how long do you keep the 70% solution before disposal? When dispensing the alcohol for filtration is it done in the same manner as it is used? E.g when using the IPA for cleaning its used in "spray bottle" and when the filtration is done, the liquid is sprayed rather than poured into the filtration vessel.

A5: I have never come across the growth of spore forming organisms in 70% IPA. Endospores are not destroyed by alcohols but will be preserved and sit there quite happily for months.

If non-sterile preparations are contaminated it could be the bulk via poor quality water or the bulk container or packaging. Recycling of sprays is not a good idea without cleaning and sterilising the bottle population regularly. Also a poor technique was an issue but now rarely used ie "dunking" items in a bath or alcohol as opposed to spraying. Once the bath was contaminated it obviously contaminates subsequent items "dunked" therein.

For those that want to have more confidence in non-sterile IPA's but dont want to go to the expense of gamma irradiated or filtered forms it is possible to make spore free preparations. Add a final concentration of 0.125% hydrogen peroxide in the finished 70% IPA solution. Studies show a spore loading of up to 1 million per ml will be destroyed after 72 hrs hold time.

From memory I also recall a situation where a thin water layer can develop in alcohol solutions (at the bottom of the container I assume because of the difference in density) where microbes could in fact proliferate.

Also there was a theory that suggested once alcohol had evaporated from the tip of a spray device the water left behind could support growth (but I never gave that much credibility).

A6: I cannot comment on the count - I am not sure we ever evaluated it. But the 70% IPA is definitely not sporidical and we have seen spores in the preparations. I don't think the Bacillus actually multiplies in the solution, but the spores that inadvertently end up in the preparation are definitely not killed, and will remain in the product if not filtered.

A7: From personal experience. This was a long time ago before there were many of the current options for dispensing IPA in clean rooms.

In my previous life in Environmental Monitoring, I was working for a company that saw a rash of bacillus recovery from clean room monitoring. After pulling our hair out looking for the source, I decided to filter and plate the IPA from one of the bottles in the clean room. Three days and one overgrown plate later, we had the source.

In this case, the IPA was filtered but they used squeeze bottles which draw in air every time after dispensing and they left the bottles in the area until they were empty. Not sure if there was a causal connection there but we implemented removing all IPA at the end of each shift and never had a problem again.

A8: i to agree with you incase of 70%IPA, But why should we do filtration/sterilization for bacillocid spl (floor disinfectant)(its a sporicidal also). could you tell me the exact reason?

GPT of sterility test media

While performing the GPT of sterility test media (SCDM & FTG), testing of single tube of prepared media for every USP test organism is acceptable or we need to perform three replicate tests for every test organisms. kindly clarify.

Bowie dick test paper & Strip

Please anyone help me to get bowie dick test paper and bowie dick test strip to perform leak test in autoclave. Himedia has stopped to prepare this test kit so please suggest me comparable manufacture for bowie dick test paper and strip specially in India.

A1: Kemlesh, Kitten Enterprises sell Bowie Dick test sheets and packs in Mumbai India, contact info is :

Kitten Enterprises Pvt. Ltd.
203, Malwa,Pantanwala Industrial Estate
Ghatkopar (West)
Mumbai, 400086
Phone: 91 22 42255511
FAX: 91 22 25001358
mapkitten@gmail.com

A2: You can get it from salesworth india ltd

A3: The Steris ones now have a zone printed on them, which provides a qualitative indication of the degree of failure (should it occur).

A4: You can procure the bowie dick test paper of manufactured by RAVEN LABORATORIES it contains 25 packs.

A5: You can contact Arvind Sales Agencies 3M Distributor.Ph.:01722569817,Fax:01723068470

media

Will the result for yeasts & molds differ if:

· I use Sabouraud Dextrose Chloramphenicol (settle plate) compared to TSA (settle plate) (but at a different temperature than the one used for total count).

Or I should expect a difference?

PLC validation

Is it necessary to go for PLC validation of the autoclave before going for its requalification?

A1: It is not necessary to do the PLC validation of the autoclave before requalification unless you donot require to change the ladder diagram.

A2: It is not necessary to do the PLC validation every time if u have already qualified it in OQ/PQ. Incase of any major repair in PLC, u need to qualify the PLC as well as Autoclave.

pulsed light for terminal sterilization

Has anyone used pulsed light for terminal sterilization? Does anyone know of a company that does pulsed light for terminal sterilization?

A1: I looked at pulsed light in the 1990's for sterilization contact lenses and IOL's and more recently in the 2000's for sterilizing aseptic pass-throughs. The efficacy of pulsed light is very good (10e6 killing of spores); however, unless the surface is contacted directly by the light (directly from the zenon lamps or reflected off of mirrors), you wont see that efficacy. Any shadows on the surfaces will not receive the optimal exposure.

Action Plan

I was wondering if someone could help me find documentation on preparing an action plan to handle any incidence of Contamination of Purified Water system by Pathogens and Distilled water by Bacterial Endotoxin, like what would be done with the system itself and Products manufactured by this water, both sterile and non sterile.

Water Testing Monograph

The EP water testing monograph has changed to state that incubation time should be 'not less than 5 days' a change from the previous 5 day incubation time.

Does anyone know the origin of the change? Does this therefore mean that we now have to state incubation times for water testing down to the hour to ensure an entire 120 hour incubation and no less?

A1: After about the 72 hr point in incubation, such as at 5 days, the difference in a few hours of incubation becomes insignificant. So I don't think the statement has anything to do with 120 hr ± some amount. I think that the "not less than 5 days" statement is there to indicate that incubating longer than 5 days is not only acceptable, but also perhaps necessary in some cases to allow more obvious colonial development. So flexibility has just been added to the upper end of the incubation period.

A2: It changed because of the arguments about R2A, which is designed for longer incubation times (although oddly the temperature remains higher than for which the media was originally designed for) This matter was discussed at the UK Pharmig conference last month, two or three companies had undertaken a study, but the majority had not.

Validation 1227 USP

I´m trying to validate one product oxytetracycline using membrane filtration with USP chapter 1227. In this formulation has oxytetracycline (217mg/mL)+ diclofenac sodium (10mg/mL), formaldehyde sodium (2mg/mL), and others. I did 10 tests, using Fluid D modified (I put Tween 80 in 0,7%, the original fluid has 0,1%, because this way the product filtered, in the beginning it was precipitating with original fluid D). I used 6x200mL + 100mL to wash the membrane, but I got to recuperate only: Pseudomonas aeruginosa, A. niger, C. albicans, B. subtilis. I don't know more alternatives to get validation this product.

In the beginning I tried to wash the membrane with 5x200mL, but I got to recuperate only Pseudomoas aeruginosa. In this chapter (USP) said to use 5 x 200mL. Can I use more cycle of wash if I didn't get this way?

A1: Recently I had the same situation with a product I am validating, as per USP 61 it Is recommended to dilute the product with a diluent (10 grams or 10 mL into 90 of diluent) that contains suitable neutralizers that will helps neutralize the antibiotic depending on the product ingredients there are a couple of examples to use, also, actually, there are culture media that will also helps neutralize these ingredients, initial assessment of product properties should be performed prior to performing method suitability testing, this will help develop a suitable protocol, it seems that your product is filtrable in this case you will only need to find the effective neutralizer (lecithin, tween 80, azolectin, etc) for you product or raw material.

A2: The USP<1227> states that "Three 100ml rinses are assumed, but the volume and number of rinses are subject to validation. Each validation run should be performed independently at least three times" so as long as you validate your product with 6-100ml x 3 you're ok.

A3: Someone knows about the MgSO4 to inactivated antibiotic oxytetracycline + sodium diclofenac in sterility test? Could you help me, because I didn´t get to neutralize this product.

A4: please try 3% MgSO4.

interpretation of AET

Can anyone guide me on the interpretation of AET results? We have performed AET on a topical preparation with different parabens as preservatives. Results showed no growth on most bacterial inoculum even at initial using phosphate buffer. We have tried using neutralizing methods and neutralizers (polysorbate and lecithin) but still we cannot arrive at desired growth with log value of 3 or higher (at initial). Although growth is slightly enhanced by use of TSB with polysorbate and lecithin, still bacterial growth cannot reach the desired log value at initial.

Would it be acceptable to base our initial count on a "placebo"? Can we use as reference point the *diluent + inoculum without the product* as our baseline for the computation of log reduction?

A1: I'm not an expert but I do not understand if you are trying to confirm the inoculum size, if that is the case, I think you'r suppose to inoculate the plates for your count directly from the buffer solution you used to prepare the microorganism suspension (no product, no neutralizer). Some analysts advised me, since the initial inoculum(use to perform the AET test) is 10E8 cfu concentrated, to perform fold dilutions until you get approx. 10E2, then plate 0.1 ml (10-90 cfu). At the time of calculating your initial inoculum size, consider your dilution from 10E8 to 10.

A2: Yes, absolutely do use a "placebo; all you have to prove is that you really did add the required inoculum. Near-instant die off will cause failure to meet acceptance criteria, so run a parallel count , diluted to countable numbers, with just growth media and neutralisers...(this will also serve to show that the neutralisers themselves do not have significant static or cidal properties).

A3: You can only base your initial counts on a placebo. I believe the methods state that you need to establish you initial counts in a saline or saline+TWEEN 80 solution. You inoculate the same volume of this blank as the volume of your sample (i.e. 10 or 20 mLs or grams) when performing the actual AET test.

The parabens are probably rapidly cidal, and it would be near impossible to establish your initial counts in them. You do need to validate the efficacy of your neutralizing media though, to show that they are efficient in recovery of the organisms at low levels (the methods are in the pharmacopoeias).

If you are unable to neutralize the product - usually filtration is your only option, which is impossible to do with a dermatological product. However, in my job a while back, we used to use Thioglycollate with Tween 80 (try 1%) and were able to validate this media with parabens.

Autoclave loading pattern

After validating the minimum & maximum autoclave loading patterns, is it acceptable to do the intermediate loads,

For example
Minimum validated loading pattern is 05 bags (1000 plugs/bag) &
Maximum validated loading pattern is 45 bags (1000 plugs/bag)

During the routine, can we do the autoclave with 35 bags (1000 plugs/bag)? If not, what is the remedy for this?

A1: The more data you have the better the validation study. Since you already did a minimum and a maximum load, then in theory anything in between should be found acceptable.

A2: You can certainly load your autoclave with any number of bags (NLT 5 and NMT 45) during routine usage, so long as their positioning is consistent with your validation.

A3: Yes, once you have validated minimum and maximum loads, you can autoclave any amount in between. The rationale is that you have tested, during the validation, the worst conditions. This is typically done in the microbiology laboratories as unlike many production loads, the contents of the loads do vary constantly.

ANSI/AAMI AT6 Interpretation?

In the process of setting up product particulate testing program for medical devices and was hoping someone could provide some input on particle counting/recording methodology/"requirements". AT6 references recordable particle levels as >10, >25, and >100um. When recording data, does this imply that a particle that is counted as >25um is also counted as a particle >10um (and since a fiber is a "particle", is a >100um fiber also counted as a > 10 and >25um particle)? Other references (ASTM, SAE) refer to particle size ranges (ie. 5-15um, 15-25um, etc) and I am not sure if AT6 is indicating to count particles from >10-24um separately from particles 25-100um and or if it is a cumulative count. Hope this is clear enough and any guidance/assistance on interpreting/reporting data would be greatly appreciated.

dark fibers on products

One of my clients has been noticing dark fibers on some heat laminated products in their CER. They have been using ionized compressed air to try to remove the fibers without success. Has anyone on this list had any experience with this issue? If so, what is the best way to get rid of it?

A1: I have seen this in the past-the fibers were actually coming from clean room wipes used to wipe mandrels. The fibers could not be seen until after the heating process where they turned black. We replaced the wipes with a more durable product and eliminated the issue.

Reporting CFU

I would like to ask your opinion of how to report CFU results. Current
practice in the lab I work is the following:

Scenario A:

Using a 10-1 dilution plated in duplicate and no CFUs are present the
results are reported as <10 CFU/ml.

Scenario B:

Using a 10-1 dilution plated in duplicate, 1 CFU is found on 1 plate the
results are reported 5 CFU/ml.

I was asked if 5 is less than 10 why are the results not all reported as <10
CFU/ml. What is your opinion?

A1: It depends what guidelines you need to follow. ASTM recommendations ask the user to report <10 CFU/mL for a 1:10 dilution if no colonies are recovered. If countable colonies are present but below the countable range, count them anyway and report an estimated count.

USP states, "Lower counting thresholds for the greatest dilution plating in series must be justified".

FDA BAM recommends that all counts are recorded in the raw data, but the information is reported out as <2.500.

In your case, you have plated one dilution in duplicate. One has a colony, one does not. Reporting <10 lets the reviewer know that there were NO colonies present. Reporting <5, Im assuming, is an average of the two and lets the reviewer know that some colonies were present.

A2: I'm guessing 10 CFU/mL is your action limit and you have a 5 CFU/mL alert limit. While the 1 CFU can be reported as <10 CFU/mL, the dilution causes you to lose the ability to determine if you are over or under 5 CFU. Since you can't tell, worst case is to report the result as exceeding the alert limit.

A3: Scenario A: the result is reported as <10 CFU/ml

Scenario B: The result is reported as <10 CFU/ml too.

If the dilution is 1:10, the quantification limit is 10 CFU/ml.

A4: I would think <10 and 5 are both correct, as 5 is <10. But you probably need to define in your SOP's how you are going to handle averaging of such data. And it may also depend on the guidelines that you are following.

In our case, we would report <10 if there were no colonies present, and reported the actual number (like 5) if colonies were present. But it was defined in our SOP's that fewer than 25 or 30 colonies per plate were not considered statistically significant or reproducible, and any low counts were recorded for information only.

We were not doing sterility testing, but AET testing for most part.

A5: I would prefer to defend reporting "<10 CFU/mL" from the perspective of the capabilities of the assay. Any decision, however, should be described in SOP with a rationale for the position.

An essay that might be of help in determining this rationale is entitled COUNTING COLONIES and can be found at http://www.microbiol.org/resources/monographswhite-papers/

A6: Here is the way I personally view your situation.

In Scenario B in which a 10(-1) dilution results with one replicate with zero CFU recovered and another replicate with 1 CFU recovered, look at it logically: A zero count from a 10(-1) dilution could actually be 0 CFU/mL, 1 CFU/mL, 2 CFU/mL... so on and so forth but you don't know for sure. All that you know is that it is a result <10 CFU/mL. The "true" count is outside the limits of your test and cannot be determined.

Now, if you look at all of the possible "true" values for your zero result and average them with the result that had detectable bioburden, you will see one thing in common with the possible averages:

Possible "true" count of Rep1 (integers less than 10), Rep2 (10)= Possible Average
9, 10 = 9.5
8, 10 = 9
7, 10 = 8.5
6, 10 = 8
5, 10 = 7.5
4, 10 = 7
3, 10 = 6.5
2, 10 = 6
1, 10 = 5.5
0, 10 = 5

The only reportable result that conforms to all of the possible averages is <10 CFU/mL. Using a "true" zero in your average disregards your dilution factor and creates a false sense of accuracy in your reported average result. Think of it this way, rather than taking the average of 0 and 1 then dividing by the dilution factor, take the dilution factor into account first and take the average of <10 and 10, which will be some unknown number, but always less than 10 on average.

The greater the dilution, the greater the error if you use a "true" 0 as part of your average. At my company we have some products that are quite inhibitory and can require up to 10(-3) dilution to overcome inhibition. It drives me crazy when the contract lab performing the test in triplicate reports a 0, 0, 0 result as <1000 CFU/g, but a 1, 0, 0 result as 333 CFU/g. This is especially troublesome if you use the data to calculate log reduction as you will get a seemingly greater reduction even though more surviving bioburden was observed.

A7: I would like to know you opinion regarding the counts upper than 100 UFC in RODAC plates to calculate the media of the results in order to stablish alert and action limits in the environemtal contamination.

A8: Scenario A: the <10 CFU/ml is your Alert/Action specification for your product.

when the enumeration testing is 0 cfu in both Petri dishes 0 cfu in dish1 + 0cfu in dish 2/ 2 x diltution 10 -1 = 0 cfu/ ml . But we always report the result < to limit specification in the case the result is 0 cfu

Scenario B:

you have 0 CFU in dish 1 and 1 cfu in dish 2 , so the average is 0.5 cfu that you multiply per dilution 10-1 = 5 CFU/mL or Gr.

Problem with String BI inoculation/stability

We are developing internal string BI's for internal purposes. We are facing the problem that we are having a one to two log reduction in population of G. stearothermophilus after a one month period. We are trying to extend this shelf life. Anyone have any recommendations? We have examined different suture/thread types. Our recovery process is effective and validated with paper BI's. Any insight is greatly appreciated.

Effect of TSB temperature on Penicillium mold

Has anyone ever done a study or know whether or not mold such as Penicillium with typical optimal temp of 25C, if it were exposed in TSB media for 7 hours at temperature of 37C that whether or not this could potentially cause the mold to die off or not recover once the TSB is incubated at 20-25C?

PET testing on Glycerol Suppositories

Can anyone advise me on a method how to perform PET on Glycerol suppositories?
BP states that rectal applications should comply with a PET testing, but there is no mention of rectal products under any of the groups in the PET paragraph. Common sense says "Do not even try to do it", but for a registration purpose I'm told it is required. Can anyone suggest a good explanation why not to perform this test?

A1: I am positive that the BP regs mentioning rectal applications only apply to items such as creams and lotions (multi-use) and not suppositories (single use.........we hope!). Who mentioned that the PET was required for registration purposes?

A2: As I suggested previously - although questionable as to why do it - we were required to test chapstick - and used to melt it down at 37C, then inoculate, stir well, allow to sit at 25C between test points, and melt it down again at 37C prior to sampling (and place into warmed up media as well). I would not go beyond 37C.

A3: I believe that this is a misinterpretation of the BP text. It might be applied to multiple use preserved products but this is hardly applicable to a suppository! In any case the practical aspects of the application of the test with even distribution of the challenge organism makes your eyes water.

A4: Suggestion for the suppository would be to argue unemotionally and document the irrelevancy. If demanded, follow the dictate of ones management or regulatory authority with the most relevant protocol one can negotiate. Short term it may present one as argumentative and unhelpful but ongoing it maintains ones technical credibility to both the above groups. I've a bias against contriving /tacitly accepting meaningless protocols.

No offense meant, [name redacted] but I take issue with the chapstick protocol. Tho it may have some remote relevance to manufacturing, it's a far cry from any relevance to consumer use. The product is multiple use and in that context has unique risks as do lipstick and even powder cosmetics - so if one must test, address that unique risk. Could see inoculating the consumer exposed surface and following the fate on bugs there. Design would have to include some expectation of efficacy and its relevance - but managing that kind of complexity is what scientists do.

A5: Who required this to be done?

A6: In the case of a suppository it would seem to me that the level of bioburden in the product would pale into insignificance when the product reached its target.

A7: This was back in the 70's, it a was chap-stick - and a multidose container theoretically. I believe it was the FDA back then that required it, plus some international regulatory agencies that we had to deal with. The dermatological criteria were regulated separately and had different requirements.

Have not come across chap-stick since then, and not sure if the regulations are the same still. I merely share the methods we used back then when someone asks how to do the testing. I take their word that someone is requiring from them - even though it often does not make sense to me either.

A8: Agree - it is hard to imagine that any bioburden, short of gross compromise of the product or active ingredient, would be significant.

VITEK 2 Compact validation

We are trying to validate the system according to the validation package suggested by Biomerieux.

There is a field named after "acceptance criteria". Can you provide any guidance on the acceptance criteria? Just to make a note that we are a pharmaceutical industry producing sterile parenterals.

We have been trying to identify Bacillus pumilus, and in specifics the strain suggested by Biomerieux, but we cannot seem to be able to identify it. Most of the times we come up with the result "unidentified". Does anyone have any similar experience? Do you have any suggestions? Can I close the validation even if I can't identify the specific strain?

A1: Can you provide more details regarding your Bacillus pumilus problems? How do you grow the bug? What media? Source of the bug? Passage history? etc? Have you tried buying a fresh isolate standard and re-tested?

A2: I don't think you can close the validation without a justification regarding the failure to identify *Bacillus pumilus*, especially if your protocol specifically lists this organism. Have you tried a different strain of *Bacillus pumilus*? a different ATCC number?

A3: Have you talked to Biomerieux? Their technical staff can be pretty helpful.

A4: Question #1
The acceptable Vitek 2 Compact identification is defined by a probability yield result of greater or equal to 85% with the confidence level of “acceptable” or better. For Validation purposes the greater or equal to 85% is an acceptable probability yield to have in your PQ and Method validation protocols since you are working with ATCC recommended microorganisms by bioMerieux. It is also good practice to include an in-house isolate per card. Perfect examples of in-house isolates are: re-occurring, investigational or failed product isolates.

Also, as a side note: at this time you should consider incorporating in your SOP clear instructions for identifications when the probability percent is near to or at 85 %; meaning the biochemistry positive results of the substrates was very low. The identification is with in your acceptable range, but is it a good identification? In some cases the instrument will provide you with instructions to perform additional test but in the majority of the cases the subject matter expert must decide to either re-test, test the sample on another instrument, run additional test or send sample out to a validated contract laboratory. Now in days with the decrease of qualified QC microbiologist in the microbiology department and lack of enforcement from the FDA to enforce <1117> (training of personnel) special instructions must be included on your microbial identification or Vitek 2 SOP for the interpretation of Vitek results at or close to the acceptance criteria. I will question Vitek results at near or equal to 85%, results at this level must be reviewed with caution. This is paramount due to the lack of “System Suitability Testing” on this particular instrument, this will unable the operator to evaluate instrument failures other than reviewing the instrument alarm log.

Question # 2
I totally agree with [name redacted] in question 2, what ever your decision must be, it needs to be justified in a deviation and properly documented in the validation protocol. Also, If I remember correctly bioMerieux validation is based on the recommended microorganisms capable of turning wells in each card either positive (+) or negative (-) regardless of the identification result. If this is the case check your results with the templates on the validation package for each microorganism and base the acceptance on the template result and not the correct identification, some times you will have a correct (+) or (-) biochemistry results with acceptable identifications. It is acceptable if your validation is based on correctly identifying the recommended ATCC microorganisms to the genus and species level. Cases with “unidentified” results must match the recommended templates and a deviation must be correctly documented and justified in your validation protocol.

Additionally check the following: Was the ATCC isolated twice? – This step is recommended by the manufacture. Lyophilized microorganisms require at least 2 passes to get required pathways running.

Are you using the recommended isolation plates?

Call ATCC and check to see if they have any complaints on this particular organism.

Are you using the required densicheck concentration for BCL 1.80 to 2.20?. Do not use MacFarland standards you must use the densicheck instrument.

Call bioMerieux to see if they have any reference to this issue.

brevundimonas diminuta

I would like to ask that brevundimonas diminuta. is it grow on cetrimide agar and how its colonial growth?

A1: Preparing Brevundimonas dimimuta liquid stock cultures for filter challenge testing can be accomplished by preparing an overnight culture in TSB (@ 30C); and then inoculating 10 mL of TSB culture into 1 L of a Saline Lactose Broth (add 7.6 g/L to a single strength lactose broth) - overnight at 30C.

I always recovered the organism using a filter membrane and placing on TSA plate for 48 hrs at 30C. Colonies of B. diminuta are yellow/beige, slightly convex, complete, and shiny. At 30C (optimum temperature) colonies are microscopic to pinpoint after 24 hours and 1 to 2 mm diameter after 36 to 48 hours.

A2: Prob. shouldn't expect to recover diminuta using Cetrimide agar.

Appl Microbiol. 1972 February; 23(2): 377-381.

Evaluation of Pseudosel Agar as an Aid in the Identification of Pseudomonas aeruginosa

Environmental Monitoring by Settle plate or Air Sampler

Would appreciate if somebody can revert back with a reference on the subject matter. Actually my question is that will the settle plate be okay for environmental monitoring of microbial contamination or regulatory agencies ask for Air sampling method as well in addition to settle plate.

A1: Active air sampling is the preferred method rather than settle plates. You can have settle plates in addition to the active air sampling for additional information. Refer to ISO 14644, USP 797, and the FDA guidelines as references for environmental monitoring methods. You will see that the industry is moving towards using active air samplers for environmental monitoring.

A2: Active air sampling can produce quantitative result that can undergo statistical analysis with less errors. Settling plates method is not perfect by no means: plates can dry out during 4 hours exposure (appropriate exposure time as per Annex 1), amount of air cannot be traced, etc)

I did ROI analysis for purchasing air samplers and it showed return on investment after 2.5 years.

a year end question

I know that is it the end of the year and you are all probably rushing to finish up projects, goals, objectives, etc. before the end of the year. Here is a question for you to ponder when you need a break.
I was recently having a discussion with someone about the training of microbiologists coming out of college. It begged the question, if you, as a microbial professional, had a choice, what types of training or information would you new college graduates to have when they appear at your door looking for employment.

A1: From my experience.....Basic Microbiology skills like Gram staining, plate reading, biochemical testing knowledge are key.

I can train anyone to do all the testing in lab but the basic knowledge of what organisms look like and smell like, how to read gram stain (and use the plate for morphology), etc..needs to be there already.

I'm a firm believer in hiring Clinical Laboratory Science folks for industry. Their skill level is superior and their multitasking skills are very good.

A2: My wish is that the new employees would know how to work and care about what they do - anything else, we can teach them if we have to. And unfortunately the colleges/universities can't teach those qualities.

A3: I'd like to see a breadth of exposure (concept and practice/lecture and lab: mycology, virology, molecular, environmental, medical micro) as well as an academic record (whether for BS or PhD) that shows discipline and focus. Also seek the less tangible capacity of vision and courage to be a scientist - tempering conventional wisdom with data and reasoning (refer to recent discussion of PET for suppositories).
I'd like to have a solid basis upon which to establish the unique technical and accept the often counterintuitive elements of our discipline.

A4: Assuming as is typical of entry-level positions, I am guessing this is a "bench" position, or "tech" if you will. In this case, I think it is useful to have a Bachelors degree in a science discipline. If you are looking for someone that will be, or develop into a "technical" or subject matter expert, then I would think its best to get a Bachelors in Microbiology as a starting point. I myself have a BA in Biology with a course in Micro, and started at the bench for a number of years and had the pleasure of working under some good people who imparted on me a great deal of knowledge. I have always personally desired I did have a Micro degree, but for the purpose of what I was doing at the time, I think basic principles and understanding in science and in micro is a great launching point. A well educated microbiologist does not make a good bench technician in cGMP in some cases, and certainly the opposite is true. Where the classically educated in microbiology has a leg up is certainly in higher level interpretation of data, developing/ implementing new procedure, troubleshooting, etc. but it is not to say these cant be taught. As I always say, I didn't learn jack in college....i learned the specifics on the job, but what I did fail to understand that I built a base of knowledge and skills that I took for granted. The complexity of your Training Program I think plays into it, and what your specific deficits are in your group. If I was running a lab of say 5 people, I wouldn't need all 5 to be at the same level of competency based on what they perform, but would like to have one technical expert with strong education in microbiology (BS in Micro, or better MS in Micro). That's just my take. Harnessing the unique and varied strengths of a variety of backgrounds I think leads ultimately to a more knowledgeable and functional group where the total is greater than the sum of the individual parts. :)

A5: One factor I haven't seen mentioned in training people for positions in microbiology is a deep understanding of sterile technique. My general observation is that it cannot be gained though taking just one microbiology course. Only through several lab courses can it become second nature. While sterile technique seems like an mundane issue, I've seen many a beginning microbiology student place a sterile pipet on the lab bench while reading the lab manual.

A6: I think that asking what kind of applied (industrial) science training someone should get in college is a bit like wishing on a star. The intention is good intention, but what's the probability of it happening.

One time, when I was in academia, a group of us were invited to the production facility of a major supplier of sterile solutions to hear about his problems. His number one problem: the kids coming out of our university had no practical skills. He had to teach them simple tasks, such as how to measure pH, how to make dilutions, etc. Since the techniques he complained about were mainly in his chemistry section, I went to the head of our chemistry department. Yup, he said; we don't care if they know how do those things. We only care if they know the book.

There is also the budget angle. Labs cost money. Colleges don't like that. Also, teachers tend to get only 0.75 contact hours/hr for teaching a lab, and it takes a lot of time (and a lot more than simple book knowledge) to get them set up and running right. So many teachers aren't too wild about them either. Add to this the bias against industry, and where's the motivation.

What lab exposure students get is being limited by the current emphasis on molecular techniques. Most compendial assays are traditional microbiology techniques; most of the rapid microbiology methods are not molecular. Most of the problem solving skills one needs in development and manufacturing/QC are rooted in understanding classic microbiology. Knowing molecular doesn't solve a contamination problem, for example.

However, even if colleges were interested in the task, just what level of proficiency do you think kids should have at graduation? "Familiar with" and "proficient in" are two different states of existence. What industry needs is GMP-grade proficiency, reliability, and reproducibility. I don't think colleges can be asked to produce this kind of skill level.

If applied training in college is not probable, what do I wish kids would get more of in college: a healthy dose of classical education. You are going to have to train them anyway, at least get someone with the potential to understand the big picture. Science must fit into the meshwork called culture. IMHO, of course.

A7: I would be happy to just have more BS, entry-level microbiologists appear at the door. It would, however, be nice to have them come with more sterilization background (ethylene oxide, hydrogen peroxide, dry heat, and steam (other than 15 minutes at 121C)), and more training in basic aseptic technique. In a perfect world, they would also have a good background in the use of general laboratory instrumentation, techniques and related theory, e.g. perform a membrane filtration procedure, use a centrifuge and calculate G forces, perform a Gram stain (w/controls and know morphology, use a UV spec and understand Beers law, understand different types of BSCs and the application of each, understand lyophilization and seed lot procedures, and similar such). Probably just a fantasy.

A8: The best question regarding the freshers, I like to suggest the few steps that may be useful:
Introduction about the Microbiologist role in Industry and their job profile
Introduction about Equipments and Techniques relevant to industry requirement.
Interaction with professional to student.
I think this may be at least support the industry to get best professionals.

A9: You have really stirred up the interests of the 'experienced' microbiologists in the industry! Question is which industry and what microbiology with what specific expertise! For any field of microbiology, be it food, environmental, clinical or plant, I would say a fundamental understanding of microbes [including bacteria, fungi, yeast, virus ..] in theory and practice and their interactions with their surroundings should be taught. Most candidates I get to screen have mostly training in biochemistry and molecular biology of microorganisms, but they lack the basic microbial taxonomy and hands on experience with bacteria, fungi, yeast etc. Some practical training/courses on various industry problems and the test requirements need to be taught. They may seem very fundamental at the course level they take along with other sophisticated advanced/modern approach of studying microbes, but in the industries we serve, we are still looking for the basics and it is very important to have fundamental training.

A10: Very true about the industrial science experience [name redacted],

I would add an important item to that wish list. I wish that schools teaching kids going into a regulated industry like pharmaceuticals would teach a course in Good Manufacturing Practices or at the least how quality systems work in our field.

As an example, I recall visiting a friend doing some grad work in microbiology. He was finishing up a study showing that when a certain bacterium is inoculated into grasses, that organism can inhibit fungal rot (which it successfully did). Having been in the pharmaceutical industry for several years already, I couldn't help but look around at the university lab he was studying at and cringing. The most blatant example, was

the autoclave chart recorder that had worn a groove into the backplate as there obviously had not been a chart or pen recording autoclave cycles since the unit had been installed (about 2 years prior). That got us talking about what GMP's and quality systems were about. I of course mentioned the obvious autoclave and added that each instrument in my lab would have to be qualified (IQ/OQ/PQ) AND continually monitored and recalibrated.

I then dropped the bombshell on his project. He had gotten the anti-fungal bacterium from a professor at another school. I gave him an example of what we would do if this project was performed in our industry.

We would ID the bacterium to confirm the species after receipt and prior to the start of the project and whether the culture was pure. My friend had not done that at all - he just assumed that the organism was what the prof had told him it was.
All aspects of the study would be monitored and recorded using qualified and calibrated equipment. I am pretty sure that the last calibration any equipment this lab saw was when it left the factory (if then).
Lastly, at the end of the study, we would confirm that the bacterium was still the same bug that we started with. This took a few seconds to sink in and kind of left him speechless. If we could not recover the initial microorganism that we started out with, it kind of sinks the project.

The last item was the spark that sent him out to look for a short course in GMP's. He is now a successful microbiologist in the biotech industry.

training courses

Can any body let me know about the courses during 2011 regarding to biotechnology for any place around the world?

Regarding the Biological indicator

Can you help me in this matter.I want to validate my 115°C cycle of autoclave for Rappaport Vassilidas media for salmonella. So can i use spore strips of B. steriopthermophilus for this validation or we have to use any different spore strips.

A1: You can use bacillus subtilis strain number 5230 supplied by SGM biotech

A2: Spore strips of Bacillus. steriothermophilus can be used for Autoclave validation for Rappaport vasilidas media.

A3: For Rappaport, temp is a bit low for stearo... 115 to 116C for 15 min may not do it for a stearo challenge but the subtilis 5230 would monitor such a cycle and still more resistant than bioburden you would find in dehydrated media for prep. I'd run the first batch with the 5230 and check for sterility. Spore strips are not for use with liquid media. The 5230 ampoules for media can be obtained from SGM BioTech.

A4: you have to use low temp BI. Because G. stereothermophilus is for 121 deg celsius and low temp BI e.g. Bacillus subtilis "5230" ATCC 35021

Antimicrobial preservatives effectiveness testing (PET) of injectable drug product

This is regarding the Antimicrobial preservatives effectiveness testing (PET) of injectable drug product.
(i) If the product is a single dose injectable & having preservatives, – whether PET to determine the effectiveness of added preservative is mandatory or not required (because the product is a single dose)
(ii) During the PET of any product, testing with actual concentration of preservative in the drug product is acceptable or we need to demonstrate the preservative effectiveness with various concentrations like 40, 60, 80 & 100% of preservative. what is the rationale for this.

A1: (i) If the product is a single dose injectable & having preservatives, – whether PET to determine the effectiveness of added preservative is mandatory or not required (because the product is a single dose)

IT WILL FIRST BE NECESSARY TO JUSTIFY THE INCLUSION OF PRESERVATIVES IN AN INJECTABLE DRUG PRODUCT. THIS WILL USUALLY BE ACCEPTABLE FOR MULTI-USE PRODUCTS, BUT WILL NEED JUSTIFICATION FOR SINGLE DOSE PRODUCTS. WHY IS THE PRESERVATIVE THERE? DOES IT WORK? IS THERE A RELATED TOXIC HAZARD?

(ii) During the PET of any product, testing with actual concentration of preservative in the drug product is acceptable or we need to demonstrate the preservative effectiveness with various concentrations like 40, 60, 80 & 100% of preservative. what is the rationale for this.

PET DATA SHOULD BE GENERATED ON PRODUCT WITH THE INTENDED FORMULATION. IT SHOULD BE PROVIDED FOR FRESH AND AGED PRODUCT. (PET PERFORMANCE CAN CHANGE WITH TIME. THIS MAY OR MAY NOT RELATE TO THE PRESENCE OF THE PRESERVATIVE AGENT - FOR EXAMPLE SOME DEGRADATION PRODUCTS CAN BE ACTIVE TOO.) CHEMICAL ANALYTICAL DATA AS WELL AS PET DATA SHOULD BE SUBMITTED IN A MARKETING AUTHORISATION APPLICATION.

Regarding wilson blair medium

Has anyone tried using wilson and blair BBS agar medium for salmonella, according to IP 2010?If yes, what are the recovery chances and what precautions to take for the media preparation.

A1: Have you taken up this matter with the media manufacturer?

expiration dates and revalidation of media

I would like to know how people are determining the expiration of in-house prepared medium and how they perform revalidation to extend expiry? Also I am wondering if sterility testing of all plated media is required for a non-sterile facility?

A1: Standard growth-support testing at manufacture, midpoint and expiry, then weekly or bi-weekly out to wherever you wanted to go, including low numbers of ATCC's and some in-house denizens, would prove what you need to prove, as long as you tested the Magic Three Lots...... as for sterility, OF COURSE it's required, even nonsterile environments have SOME standards and if your media's growing things all by itself how do you know what your environment's contributing?

A2: We have quite an extensive media control program in-house at my lab and I'll try to address a few of your questions:

Expiry dates of in-house prepared media.

I would start with some references. A good place is Scott Sutton's white paper at this link: http://www.microbiol.org/resources/monographswhite-papers/quality-control-of-microbiological-culture-media/
I would run beginning middle and end studies of your expected expiry dating. A good starting point for setting those dates are using published holding times such as Table 9020:IV. Holding Times for Prepared Media in Standard Methods for the Examination of Water and Wastewater and the published hold times from media manufacturers.
Several parameters that will need testing is growth promotion, pH, moisture loss and of course sterility.
If these parameters pass at the last time point, then you can begin to try to extend some of those times, performing the appropriate tests at the new hold times.
Test at least 3 different lots if at all possible

Document everything so that when regulators or auditors ask where you have come up with those dates....voila, there they are.

As for sterility testing, we run negative controls (sterility testing) for every medium we use during the test. If any of those fail, that calls into question the test results. This covers two areas, the sterility of the medium and the competency of the technician.

A3: I would recommend that you check the pH and conduct microbial growth promotion studies of the microbial media and compare the results from when it had been prepared after the initial date of preparation. If you are trying to extend the expiration date of microbial growth agar in Petri dishes or in a non-screwed capped containers, I would suggest that weight studies be included as part of the test criteria due to the possible evaporation of water from the media in Petri dishes and non-screwed capped containers. I would also include the incubation of some of the stored media to see if the media has remained sterile no matter if the facility is a non-sterile or sterile manufacturing facility. The purpose of conducting the incubation of microbial growth media is to demonstrate that the integrity of the media container is still intact to show that it is still sterile.

sterility testing

I'm interested in finding out what sterility approach is more common for medical device/pharmaceutical companies:

1. Are companies performing sterility testing on final products after terminal sterilization?

2. Or, are companies releasing final product based on passing sterilization load biological indicator results?

contract testing lab

Can anyone provide me with the name of a contract testing lab that would be capable of validating a chemical sterilization process per AAMI/ISO 14160-1998?

Use of solvents

I was wondering if anyone has experience using solvents as a diluent when performing microbial limits test by membrane filtration? Specifically acetonitrile (10% or greater), methanol, or DMSO? Were you able to demonstrate absence of toxicity when performing suitability? Did you add the organisms directly to the blank prior to filtration?