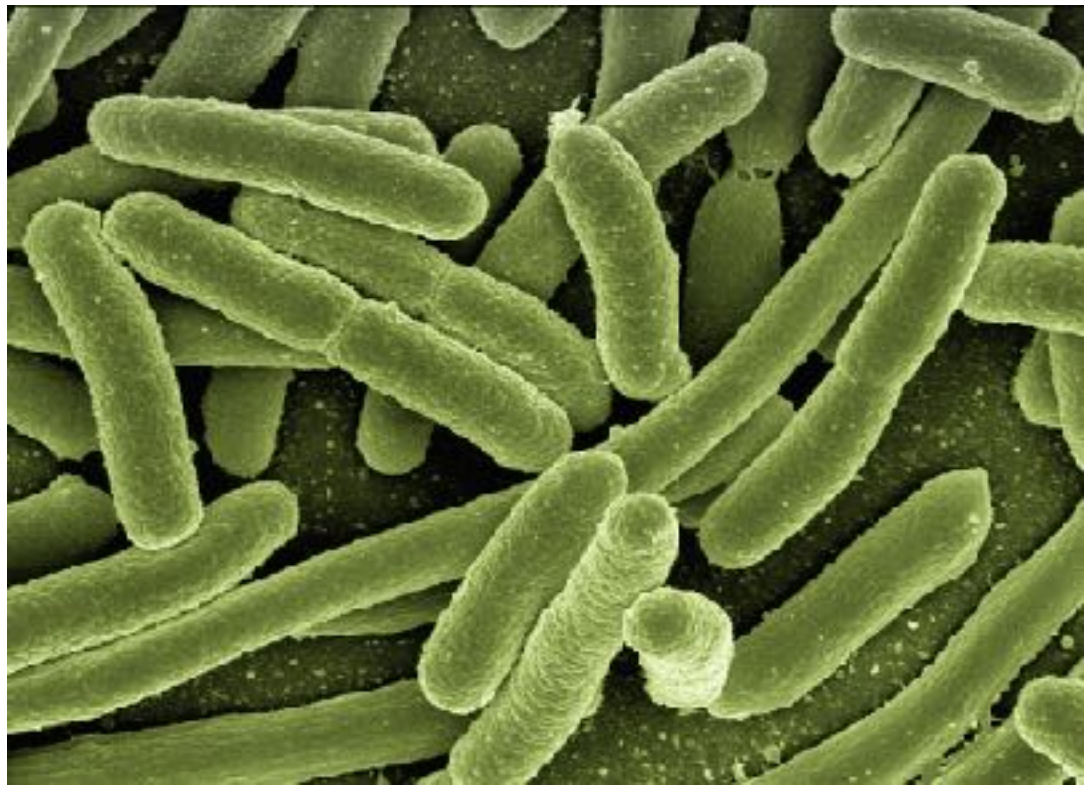
PMFList Yearbook

2021



Topics

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 2021. 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 2022 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.

Contact plate sampling pressure/duration

Most of the devices that I have seen from Millipore and Biomerieux that mechanically perform the surface sampling test using contact plates seem to use a pressure of 0.5Kg and a contact duration of 10 seconds. Is there a reference for the origin of that pressure and contact time specification?

A1: I'd like to see where that came from as well. I'd wager that if I used 0.3 kg/cm2 (mind you, 0.5kg is a weight and not a pressure value) for 2 seconds I would get similar results. Do you also need a calibrated timer to ensure contact time (not sure if one is built in)?

The action of picking up an microorganism from a surface is that of adhesion to the agar surface and not motility of the microorganism.

Not a fan of those silly weighted devices as they will need to be disinfected or sterilized for each use and calibrated. More trouble than they are worth.

You perform recovery studies to demonstrate the effectiveness of your sampling technique just the same.

A2: This is not a high precision measurement and attempting to control the sampling parameters is not worthwhile.

The Standard Methods for Examination of Dairy Products 13.044 RODAC Plate (Agar Contact) Method offers the following instructions Procedure: Remove the plastic cover and carefully press the agar surface to the surface being sampled. Make certain the agar meniscus touches the surface and use a rolling uniform pressure on the back of the plate to effort contact. Replace the cover and incubate the plate in an inverted position for 24-48 hours at 32 degree C.

A3: You can find these references (Contact plate sampling pressure/duration) on ISO 18593:2018(E)

A4: Interestingly in the recently revised FDA ORA "Pharmaceutical Microbiology Manual" ORA007 the following is given as the suggested Rodac technique:

Gently but firmly touch the RODAC agar surface against the area being sampled, exert moderate, even, vertical pressure and then carefully replace lid. Avoid using rubbing motions of the plate at the sample site as this may break the agar.

A5: You are correct that ISO 18593: 2018 (E) - Microbiology of the Food chain – Horizontal Methods for Surface Sampling contains this information. However, it should be noted that this document specifies horizontal methods for techniques using contact plates, swabs, sponges, and cloths on surfaces that are in a food chain environment for detecting and enumerating microorganisms. It is stated in the document that there is scientific literature that optimal results for contact plates are obtained by using a contact time of 10-seconds by using a pressure obtained with a mass of 500 g.

However, there is no provided scientific reference in this document for a contact plate surface sample by using a contact time of 10-seconds with a pressure obtained with a mass of 500 grams. However, it should be noted that there are several scientific suppliers that offer a Contact Plate Applicator that utilizes a stainless steel 500-gram weight and a calibrated built-in precision 10-second timer to take contact plate surface sample.

I will admit that I have never seen anyone use a Contact Plate Applicator to apply a contact plate to a surface. In general, the microbial recovery by using a contact plate is less than 50%. I wonder whether using a Contact Plate Applicator when applying a contact plate to a surface would increase the level of microbial recovery.

A6: I agree with other responders. Trying to use such more precision device (weight pressure and specific time) to sample by contact agar plate would be something like using a 0.01 sec precision stopwatch to measure a timeframe of "about an hour ".

Heat killed Lactobacillus

Today's query comes to us in the form of the manufacture of an active + probiotic capsule. The probiotic material would be heat killed lactobacillus. Do you or do you know of anyone who has experience in using probiotics in their site and how they are evaluated from a cleaning perspective? As it is a bacteria I would expect there is a micro element to the cleaning verification but do you know of anything else? How it will affect the environmental monitoring? This is new for me.

A1: [name redacted], a heat-killed probiotic makes no sense as the benefits are through live cultures.

That aside for a cleaning validation study you would use the selective medium and incubations appropriate for the specific lactobacillus and use TSA for routine monitoring.

Unless you have biofilm formation bacteria are removed by physical removal like a chemical residual. You could use TOC measurement as a measure of cleanliness.

A2: I thought the whole aim of a probiotic was to..
First survive the gut
Second maybe to colonise and proliferate in the gut.

But that aside I would start with ensuring and validating the heat killing process. Ensure thee are no survivors, if injured but still living Lactobacillus get into the environment they could contaminate your process and you would not know it.
The cleaning process could then focus on either the internals of process equipment or on cleanroom surfaces.

In both instances the removal of process residue viable and non-viable is the aim. So you would have a standard for organic residue eg TOC and a standard for viable particles via contact plates, swabs etc per unit area of surface. Air monitoring would also be appropriate. Micro organisms per unit volume of cleanroom air.

A3: I’m in agreement with [name redacted] about why you are using a heat killed Lactobacillus strain in a probiotic product since all of the benefits are from a live culture. Instead of using a live culture, are you using an extract that has been derived from a Lactobacillus strain instead in your probiotic product? If so, I can understand as to why you would a heat treatment to kill off live cells to have reduced counts.

A4: We are a contract manufacturing, this is requested by a customer to use "heat killed" Lactobacillus, I don’t know about the effectiveness of the product but from google search it seems like the content of dead cell (DNA and protein) by digestion will trigger some immune respond and act as a probiotic.

My question was if we are going to manufacture with heat killed Bactria, which kind of validation testing will be required and most importantly would it have any impact on our environment. The supplier will heat the bacteria up to 115 C for 20 min to kill them off so I know there won't be any chance of proliferation within the manufacturing environment.

A5: OK let's put aside the question of the efficaciousness of killed Lactobacillus.

My experience is Gram-positive rods will be bacterial corpses after steam sterilization. Under oil immersion magnification you will see the outlines of cells but proteins and nucleic acid will be denatured.

The issue is it is unlikely that a CMO will use dedicated process equipment so it comes down to a clean validation question. A cleaning process that removes chemical residuals will likely physically remove bacterial corpses.

A6: If the Lactobacillus has been killed, it should not pose a risk to your manufacturing environment. I would recommend that you at least conducted a microbial count of the killed Lactobacillus raw material to show that there are no survivors by using MRS Agar with an incubation period of 48-hours at 37C. Any recovered colonies should at least be identified to the genus/species level.

I think that your customer is using the following article for justification as why they are using killed Lactobacillus cells in a probiotic product due to potential safety concerns about using live cells:

Pique,N., Berlanga, M., and Minana-Galbus, D.
Review: Health Benefits of Heat-Killed (Tyndallized) Probiotics: An Overview.
Int. Journal of Molecular Sciences, 2019, 20, 2534.

From my experience with probiotics, I have never seen anyone use heat-killed cells, but I do understand there reasoning that the dead cells would provide the same benefits as live cells.

Which lactobacillus strain used in manufacturing of Pessaries.

I am searching lactobacillus species for manufacturing of Pessaries but I couldn't specific strain can you please help to guide for below questions.
Which lactobacillus strain used in manufacturing of Pessaries.

And why these strain is used.

Also please suggest industry practice.

A1: I believe that you have not received a response to your posting because most people are not familiar with this type of product formulation. I will admit that I'm not that familiar with your type of probiotic product formulation.

It is my understanding that a pessary is a prosthetic device that is inserted into the vagina. Furthermore, it is my understanding that lactobacilli in a pessary is used in the treatment and prevention of microflora disorders of the vagina.

From www.clinicaltrails.gov, it is my understanding that there was a clinical trial being conducted in 2019 and 2020 in which the sponsor of the study was Hartford Hospital and the collaborators were the University of Maryland and the University of Conn. This study was using a mixture of L. crispatu, L. gaseri, L. jensenis and L. rhamnosus. The results of this particular clinical study has not been published yet.

However, I have also seen a 2020 publication (Zhana Poyac et al. Ceska Slov Farm) in which L. case i had been used in a pessary.

Based upon various published articles, there is no absolute proof that using lactobacilli in a pessary has a clinical benefit because studies have not been stringent enough to demonstrate a clinical benefit.

I would suggest that you conduct a scientific literature search to see what Lactobacillus strains are being used in pessary product formulations.

A2: Pessaries are medical devices to block or support the vaginal tract. Although lactobacillus is a major part of the microflora that you could isolate and characterize, how would this work?

Different Chlorine Efficacy on S.aureus and MRSA

I am using both *S. aureus* and *Methicillin Resistant S. aureus* (MRSA) bacterias for the efficacy comparison of powder and liquid chlorine (Calcium and Sodium Hypochlorite).

I would have expected same efficacy with these two disinfectants for both microorganisms. However, there seems a difference between their resilience. (For example, Calcium hypochlorite is more efficient for S. *aureus*, whereas the more efficient disinfectant for MRSA is Sodium Hypochlorite).

I couldn't find any article comparing S.a. and MRSA against chlorine. I am just curios if anyone had experience or have any knowledge regarding this topic.

Raw Material and Microbial contamination

Downstream manufacturing team identified three new raw materials as starting material for preparation a buffer solution. Three raw materials as follows:

* Diluted Acetic Acid (1N)
* Sodium Acetate Trihydrate (USP & Ph. Eur.)
* Benzyl Alcohol (USP & Ph. Eur.)

Incoming tests and specification will be established per compendia monograph. Please advise for the microbial contamination tests and specification. Which tests would be the right test per EU GMP Annex 1 : Sterility, Bioburden and Endotoxins.

A1: I would suggest check Monographs as applicable for your firm. If it is available you can perform suitability study and use accordingly. If it is not available, I would suggest performing Endotoxin testing with Risk assessment .As Sterility / Bioburden will provide you assurance of Qualitative and Quantitative estimation of bioload which will be eradicated on sterilization.

Question regarding monitoring of a Decontamination Shower for High Potent Compounds

We are currently in the process of building out a high potency product cleanroom facility. The facility is currently designed with a Grade C cleanroom which the operators must exit through a RODI-supplied decontamination shower (essentially a pass-through with sealed doors while the shower is operating) and then step out into another area. The current design is roughed out below. We are currently experiencing some arguments with our Facilities Team regarding the classification scheme and my questions are:

1. Is anyone familiar with industry guidelines regarding whether the decon shower can be left as Unclassified even if the rooms on either side are classified?
2. Is it reasonable to not perform monitoring in the shower since there is no air flow? Or could surface samples be taken instead of airs/non-viable particulates?
3. It seems problematic to hold the decon shower room to Grade C limits, but is it reasonable to have the cascade go from Grade C to Unclassified Decon back to Grade C or Grade D?

Any familiarity or guidance with this would be greatly appreciated!

A1: I do not have much experience with decon showers, but industry standard is a step-wise progression for cleanrooms. Is the proposed airflow from the cleanroom to the shower, or from shower to cleanroom (i.e.: contamination control effort to prevent the highly potent compounds from leaving the Grade C cleanroom)? If the former, this is likely defendable with a robust EM program on either side of the shower and robust procedural controls (is there standing water? Do operators have to remove it? What happens with wet gowning? What is the cleaning program like? Etc.). If the latter, it will be trickier to defend in my opinion.

A2: In looking at your questions, it begs the question, which regulatory bodies does you company claim compliance with?

A3: We are currently regulated by the FDA (currently marketed only in the US), but we will most likely be looking to expand into Europe and the UK in the future. It's my understanding that the current design is that the rooms are pressurized to contain potent compounds (so from shower to cleanroom), but there are sealed doors during the actual decontamination shower and personnel flow is one-way out. We will have to obviously have procedural controls in place (as Liz notes in her response), but we haven't gotten that far yet.

A4: I would like to suggest , assess Decon shower area w.r,t Risk towards product quality by using HACCP approach.
if Risk is higher you have to monitor the area according to Grade C controls . If product risk is lower you can monitor the area for Information purpose and later period you can derive your limits based on the statistical analysis.

In addition to this,
1.Do you temperature/ Humidity control in the Dec shower area?
2. Do you find any Stagnant water in the Decon shower area?

A5: Based on EU Annex 1 I can’t see a way to justify having an unclassified room between two classified rooms. It seems like making the shower room and the adjacent exiting room Grade D would make the most sense.

A risk assessment addendum would probably be the best way to write up the justification and rationale for the classification decision. I would do particles and viable air of the static room to qualify and then use surface monitoring and periodic (maybe monthly) static particles and viable to ensure the area can be adequately cleaned and remains in compliance to meet the room classification.

A6: I would suggest you review this with MHRA as in the annex for sterile processing, the grades are sequential A->B->C->D->C->B->A There are provisions for staying the same level, but i believe there may be some that question the non-classified area.

FW: BIs used for 115.5 sterilization cycle

Is anyone aware of a vendor that will provide a BI suitable for use in a 115.5 degree C, 40 minute cycle for terminal sterilization?

A1: An possible biological indicator to search for is Bacillus coagulans. A noted expert for this BI is Dr. Jeanne Moldenhauer, who is a frequent writer to this Forum.

A2: if you know the D-value and Z-value of candidate BIs you can make an informed selection for the cycle temperature and duration you want.

A3: I have sued *Bacillus smithi (formerly known as Bacillus coagulans) with 115 deg C cycles in the past.

Viable Sampling Locations in Cleanrooms

In ISO 14698 there is a requirement that a settle and contact plate shall be used at each sample location. What is the reason for this? I work for a contracted medical device manufacturer and due to Kaizen events, clients coming and going, it has become a challenge to sample consistently the same location because the table could move or would not be utilized by operations. To combat this each client's project was environmentally validated (needless to say we take many more samples than required).

When I environmentally validate a project, I take a sample (both air and surface) at every work station for 5 days (also one day at-rest). The locations that yield the highest results are chosen for routine monitoring. If location #1,5, and 9 yielded the highest for surface but locations #1,6, and 10 yielded the highest for air, those locations would be chosen and identified on a sample layout whether they are surface or air samples. Based of the surface area of the project and how many operators are on the manufacturing line, as the surface area and operators increase so does the risk and sample quantity/sample frequency. Let's say for this example you should have 3 sample locations.

From an auditing standpoint, would this be acceptable?

A1: I couldn't find the reference inside ISO 14698 mandating a surface and air sample at every location. I know each facility needs a sampling plan that is usually a mix of surface, air, personnel and water samples. This EM plan must be based on the risk assessment of the each facility. I think your plan of measuring, then choosing the sites for sampling is fine as long as it's based on risk, which it sounds like you're doing. This is what I saw in ISO 14698 that seemed to be most appropriate:

[cid:image001.jpg@01D6EA56.72239570]

BCC in powder for oral solutions

Our product is dry powder form for oral suspension packed in sachet and it is reconstituted by end user as per the instruction given on label and immediate use after reconstitution.

We performed the test for total enumeration and test for specified microorganism (E.coli, S.aureus, P. aeruginosa and Salmonella spp.)

As per USP <;60>, BCC test is required for aqueous preparations for oral product.

Query:

Our one of the product is not aqueous preparations however Is it required to include specified organisms BCC in specification?

A1: Based on the information you supplied I believe the specification should be TAMC NMT 10^3 cfu/g , TYMC NMT 10^2 cfu/g and absence of E.coli in 1 g.

A2: No, not required, it's applicable for liquid preparation.

Pure steam generation validation

Please let me know for Pure steam generation system qualification how many days to do sampling and testing, what are parameters to be consider for qualification and please refer guidelines.

Endotoxin Testing for Propylene Glycol

I'm curious to know if anyone has experience with testing Endotoxin for Propylene Glycol. Our current testing approaches have resulted in no reactions.
I believe due to the viscosity of this material their may be masking. I'm interested to know if anyone has any suggestions in regards to diluents, dilutions, suggestions etc.

A1: Propylene glycol is used as a solvent or co-solvent in parenteral products at concentrations ranging from 10 to 60%. It is produced by chemical synthesis and is unlikely to be contaminated with bacterial

endotoxins. It can be autoclaved and has moderate antimicrobial activity. There are no bacterial endotoxin specifications in the USP/Ph.Eur./JP.

If you must subject it to bacterial endotoxin testing, I would test a 1-10% solution in WFI.

A2: Unfortunately I don't have anything specific to Propylene Glycol. My recommendation would be to do a characterization or Inhibition/Enhancement test by simply testing a series of dilutions with LRW from 1:10 to 1:10,000 to ascertain where interference is overcome and then maybe go back and run some smaller dilutions around that point. Going back would depend on if you really needed to for MVD, assuming you have some endotoxin limit in mind. This is where you should start with any sample you don't have a history with as you should have this cartelization test on file. I'm pretty confident that it's not going to take anything outside of dilution with LRW because problematic samples are pretty well known at this point and I don't recall this being one.

What is Actual Product quantity for microbial limit test

My question is that can I use 10 grams products Quantity For total microbial count and For Pathogen Detection including salmonella and shigella please suggest.

Change in material or component

What is the expectation or requirement to repeat method suitability for a raw material or component if you change the supplier or they make a change in the process or perform some type of paper risk assessment?
Also, what about the finished product in container and need to repeat method suitability?

My concerns are related to if there are different impurities or residual chemicals from the manufacturing process of materials or if a different lubricant etc are used in a pMDI valve. Could these have an impact on recovery and must be proven?

A1: I assume you mean repeating the method suitability testing required to a compendial microbial test of a pharmaceutical ingredient.

The expectancy is that a new vendor, change in manufacturing or ingredient specification would go through formal change control .If the ingredient has a USP-NF monograph and you change supplier you may consider this a like-for-like change and the method suitability would not be repeated. If there are significant changes to the test material or the test method you would repeat the suitability testing.

In the 2006 FDA Guidance For Industry - Quality Systems Approach to Pharmaceutical GMP Regulations states:

Change control is another well-known CGMP concept that focuses on managing change to prevent unintended consequences. The CGMP regulations provide for change control primarily through the assigned responsibilities of the quality control unit. Certain major manufacturing changes (e.g., changes that alter specifications, a critical product attribute or bioavailability) require regulatory filings and prior regulatory approval (21 CFR 314.70, 514.8, and 601.12).

A2: For a different supplier, I would recommend a separate method suitability to confirm the method.

A3: I would think that a comprehensive risk assessment would allow you to make those decisions and be very useful to support / defend your decisions in future. Key would be getting the right people involved at this stage to make sure it is a robust document.

Media Fill Quantity Question

Per the FDA Guidance for Aseptic Processing, the size of the media fill should “mimic commercial production conditions and accurately assess the potential for commercial batch contamination”… “A generally acceptable starting point for run size is in the range of 5,000 to 10,000 units”. There is also direct verbiage for fills at or below 3,000 units that the media simulation should equal to that of commercial batch size.

We have a typical batch size of 24,000 lyophilized vials (12,000 per lyophilizer) that are filled and lyophilized in a continuous Grade A isolator.

I understand the reasonable answer is, whatever your risk assessment says but do any of you have any recommendations? Do we need to do the full 24,000 or is 10,000 (5 through each lyo) sufficient?

A1: 10,000 should be sufficient as long as they are filled for the duration of a normal run, e.g. 36 hours.

A2: 10,000 vials will be enough for you. We have the same situation for lyo. We also fill 10,000 pieces.

A3: The filling time will be the same but we will not include the full lyo time (7 days). The vials will just move into the lyos to mimic the movement and then move out to the next step.

A4: It is fairly common to use the 10,000 vials in this case. Additionally, you might look at placement within each lyo (top, middle, bottom) as part of your matrix.

Need Opinions on Aseptic Processing

If a mechanic needs to open a panel or panels underneath the work surface, do you require a full re-sterilization of everything and room disinfection? If so, after what period of time the panels are open?

Have you performed a media fill to support this? If so, for what period of time or # of units?

Following The 1% Rule in USP 61

We are currently setting up the procedure for our bioburden suitability testing based on the rules found in USP <61>. We are doing to the pour plate method and for our sample, we will be diluting 10 mL into 90 mL of buffer to make the 1:10 dilution. Then, 1 mL of the diluted product would be plated. We are targeting 10 - 100 CFU counts on each plate per USP <61>. However, we are running into a problem following this rule: the volume of the suspension of should not exceed 1% of the volume of the diluted product.

Many commercially available kits provide 10 - 100 CFU per 0.1 mL spike. If I spiked the 1 mL of plated diluted product with 0.1 mL to achieve 10 - 100 CFU on my plate, then the volume of the suspension is 10%, violating the 1% rule mentioned above. However, if I spike 10 mL of the diluted product with 0.1 mL to make sure we are following the 1% rule, when I go to plate 1 mL of it, we will only get a count of 1 - 10 CFU on the plate, not the 10 - 100 CFU we are targeting.

How do others use these kits that dispense 10 - 100 CFU per 0.1 mL spike while also following this 1% rule? Thank you for any insights!

A1: I believe you are confusing the volume restriction in USP <51>
Antimicrobial Effectiveness Tests with the pour plate procedure in USP <61>.

A2: You must use 10 microlitre of < 10000 UFC ... You cal have 10 - 100 UFC in every plates.

A3: Because you are doing pour plate then as you say your 0.1ml containing <100cfu is 10% of your 1ml. If you were doing filtration method and could filter the full 100ml or 10ml (product and diluent) then you would be fine.

You need to add your inoculum into your 100ml (product and diluent mix) (or maybe you have made 10ml aliquots to use for your different organism inoculums). You would need to add <10000cfu in to the 100ml or <1000 in to the 10ml (for example in 0.1ml inoculum) and this would work out as <100cfu per 1ml that you would put in your petri dish for the our plate, giving you your countable range.

Your supplier will most likely also supply higher count organisms that they should have for AET (100000 to 1000000) and you can perform some serial dilution to obtain your higher inoculum count for you 0.1ml addition.

Disinfectant revalidation

How often do you revalidate your disinfectant efficiency, for clean rooms?
Annually or less?
Is there any justification for the revalidation times?

A1: The USP chapter is currently undergoing revision and is a good source of guidance.

Re-validation would only be necessary if you changed the disinfectant supplier.

It is recommended that the most frequently isolated microorganisms from an environmental monitoring program be periodically subjected to use-dilution testing with the agents used in the disinfectant program to confirm their susceptibility, because there may be real differences in resistance among different species to the lethal effects of different agents applied to different surfaces.

A2: I typically recommend assessing the cleanroom bioburden from your environmental monitoring program on an annual basis. If there is a new mold or bacteria that frequently shows up or appears to be hard to kill then I would conduct coupon testing against the new strain. I would assess all of your disinfectant coupon studies every 5-7 years and if there are any issues with controls or log reduction numbers you may want to consider repeating those coupon studies. If there are new surfaces in your cleanroom you would want to include those in your risk assessment for your coupon studies as well.

A few useful references are USP 43 <1072>, PDA's Technical Report #70, and Draft Version 12 of Annex I.

A3: The FDA and EMA expect cGMP facilities to continually evaluate their disinfectants use efficacy. The idea is that your facility will have a robust EM plan and the current common isolated microbial agents will be added to your evaluation process. There is no indication of microbial resistance and therefore the justification for repeating the same set of experiments is somewhat unfounded. However, you can always improve your model by using more representative organisms and surfaces - worn out Stainless steel and not bran new for example. Improve your model by adjustments to the application methods and account for additional surfaces not covered initially (uPVC, other materials in the facility etc.). Over time, formulation may change (see current LpH and Vesphene), requirements (draft Annex I), mops, sprayers etc. Rarely nothing changes over time and is identical to the practices in place a decade ago. In short, regulators expect you to be vigilant and not rely on really old data.

These are large studies that are both expensive and may take years to complete. I would recommend revisiting the data every 5 to 7 years (an official Risk Assessment will be the way to go).

Disinfect- Use dilution testing for frequently isolated microorganisms

As per USP disinfect chapter, It is recommended that the most frequently isolated microorganisms from an environmental monitoring program be periodically subjected to use-dilution testing with the agents used in the disinfectant program to confirm their effectiveness.

My question is - How use-dilution testing is performed on frequently isolated microorganisms? Is it tube dilution method or Surface challenge method?

A1: If you read USP <1072> you would be able to answer that question.

The use-dilution test is conducted by inoculating the disinfectant in a tube.

A2: The efficacy of disinfectant in solution against resuspended microorganisms is substantially different than the disinfectant efficacy against the same microorganisms when those are deposited on a surface and allowed to dry prior to the exposure to the disinfectant. There are several contributing factors at play. First, surface chemistry is different then the bulk chemistry in solution. The diffusion is reduced as is the microbial surface area exposed to the liquid. There are also possible interactions with the surface itself (depending on the type of surface). Lastly, with the exception of Spores, there is a stress response taking place while drying and/or attaching to surfaces the microbial entity goes through. To put it in perspective, it is like the difference between a surprise attack and trying to mount the same attack a week later. The second time is much harder.

I will shy away from solution based studies as this model is far from ideal. Not that coupons are a 1to1 exact model system but they are much more informative and realistic.

A3: As per USP 1072 tube dilution test is preferred method. However, if you look 483' inspector had given for DET not performed by surface challenge method hence better to perform study by both the methods.

A4: I would comment on the last two posts.

Firstly a 483 observation is the opinion of the FDA investigator and may not be technically sound.

The role of an use-dilution test is to confirm on the basis of a relatively simple laboratory test that the organisms isolated in your monitoring program are susceptible to the disinfectant.

A5: We perform analysis of our environmental monitoring isolates and pick representative isolates (appear multiple times across different areas and at different times).

Once we have these isolates we test them in the coupon method (yes submerging the isolate in the disinfectant is easier - but is it representative?). They can substitute counterparts from the USP list (if you use spores of an environmental bacilli you can stop testing B. subtilis - same for Staph.

The USP clearly states: "The selection of suitable disinfectants and the verification of their effectiveness in SURFACE CHALLENGE testing is critical in the development of a cleaning and sanitization program."

I think that when you screen which disinfectant to use - it make sense to use the dilution method - but after you establish conc. and contact time then the way to go will be coupon testing.

Your reading of the regulation may be out of context - as the dilution method is mentioned in the following paragraph:

"In the United States, the official disinfectant testing methods are published by AOAC International3 and include the Phenol- Coefficient Test, Use-Dilution Method Test, Hard Surface Carrier Method, and Sporicidal Carrier Test. A scientific study submitted for EPA review in support of disinfectant registration must be conducted at a laboratory facility that follows the Good Laboratory Practices (GLP) regulations (21 CFR 58). To demonstrate the efficacy of a disinfectant within a pharmaceutical manufacturing environment, it may be deemed necessary to conduct the following tests: (1) use-dilution tests (screening disinfectants for their efficacy at various concentrations and contact times against a wide range of standard test organisms and environmental isolates); (2) surface challenge tests (using standard test microorganisms and microorganisms that are typical environmental isolates, applying disinfectants to surfaces at the selected use concentration with a specified contact time, and determining the log reduction of the challenge microorganisms)"

and the description of the surface test method soon follows:

"The disinfectant efficacy test must have realistic acceptance criteria. In practice, sufficient organisms need to be inoculated on a 2-inch × 2-inch square of the surface being decontaminated, i.e., a coupon, to demonstrate at least a 2 (for bacterial spores) to 3 (for vegetative bacteria) log reduction during a predetermined contact time (i.e., 10 minutes over and above the recovery observed with a control disinfectant application). The efficacy of the neutralizers and their ability to recover inoculated microorganisms from the material should be demonstrated during the use-dilution or surface-challenge studies. Points to remember are that disinfectants are less effective against the higher numbers of microorganisms used in laboratory challenge tests than they are against the numbers that are found in clean rooms (see Microbiological Control and Monitoring of Aseptic Processing Environments á1116ñ); that inocula from the log growth phase that are typically employed in laboratory tests are more resistant, with the exception of spores formed during the static phase, than those from a static or dying culture or stressed organisms in the environment; and that microorganisms may be physically removed during actual disinfectant application in the manufacturing area."

color in capsules

I have a question about the color used in hard gelatin capslue manufacturing.
If hard gelatin capsules are made with different colors (one capsule is yellow, one is red for on other non sterile finished product) is necessary perform different suitability or is it possible to combine them all in one validation method.
I was doing bibliographic research but I can't find if the dye used in the production of hard gelatin capsules can have antibacterial / antifungal activity or not.

Do you have any experience about this question?

A1: Given that hard shell capsules are prepared from molten gelatin and are low bioburden material why are subjecting the capsules to microbial testing.

A2: We perform microbial testing because our hard shell capsules maker perform test at each batch of raw material that we buy. We have a skit test on finish product (we perform one microbial test every 10 batches of products), in these case our company want perform the test to assure the incoming raw material.

The question has arisen about the color used to make the capsules during our internal meeting (if there is antibacterial / antifungal activity or not that can interfere with the suitability) and I have not enough data or information about this question (even if I know the low water activity and low contamination risk of our product and of capsules as raw material).

A3: Back in the day (about 15 years ago) I did test gelatin capsules. My experience with some dyes in oral solide dosage was that they could be inhibitory, therefore, we would perform a screen validation when tablets changed colors. These were used in clinical trials so the tablet colors were often changed.

A4: We do the same. And we have seen some colors more inhibitory than others. The agency has requested this testing for the most part of our clients and we do occasionally see some contamination. Most of the reputable capsule suppliers however are normally very clean.

A5: I've also seen contaminated capsules, especially if they are exposed to moisture.

A6: I have also seen the same thing that you had seen in which some dyes or colorants will have antimicrobial activity. For example, I have seen from my experience that FD&C Red No. 3 (Erythrosine) will have antibacterial activity, but there was no antimicrobial activity for FD&C No. 3 (Fast Green) and FD&C Blue No. 1 (Brilliant Blue). I'm sure that there is a literature reference showing that Erythrosine having antibacterial activity. To do a proper literature search in determining whether a colorant has antimicrobial activity, you cannot use the FD&C number or Trade Number. You need to use the actual

chemical name of the dye to do the scientific literature search. However, I'm not sure if there are other FD&C dyes or colorants that have antimicrobial activity which are being used in tablets and capsules. With colorants, I have found that the biggest problem with microbial contamination comes when color solutions for capsules are not immediately used in manufacturing after preparation and are stored for several days at room temperature in which microbial proliferation will occur. Furthermore, I have also seen filled capsules become contaminated with mold growth even with the presence of a desiccant silica packet that is used to remove water vapor from packaged containers.

I am not surprised to see that the agency had requested companies to perform microbial content testing on capsule products. If a colorant or dye has antimicrobial activity, this inhibitory activity should be detected during suitability testing of the microbial content test methods in which Tween and Lecithin will need to be used to overcome the inhibition.

Propylene Glycol

There have been several postings about performing endotoxin testing on propylene glycol.

It should be realized that there are several chemical production methods that can be used to produce propylene glycol. For the most part, propylene glycol is mainly produced from propylene oxide which can be derived from either petroleum or natural gas. By using either a non-catalytic high-temperature process at 200 C (392 F) to 220 C (428 F) or a catalytic method, which proceeds at 150 C (302 F) to 180 C (356 °F) in the presence of ion exchange resin or a small amount of sulfuric acid or alkai, propylene oxide is converted into propylene glycol. By using these 2 chemical manufacturing processes, the final product will often contain 20% propylene glycol, 1.5% of dipropylene glycol <https://en.wikipedia.org/wiki/Dipropylene_glycol>, and small amounts of other polypropylene glycols <https://en.wikipedia.org/wiki/Polypropylene_glycol> Additional purification steps are performed on this intermediate material to produce to finished industrial grade or USP/JP/EP/BP grade propylene glycol that is typically 99.5% or greater in concentration.

Besides using propylene oxide, it should also be noted that propylene glycol can also be obtained from plant-based glycerin. To convert plant-based glycerin into propylene glycol, a catalytic process called hydrogenolysis is used to convert glycerin into propylene glycol. A reaction between glycerin (obtained from vegetable oils) and hydrogen takes place at high temperatures and pressures to produce a reaction that forms propylene glycol.

Due to sustainability reasons, I do know that there are now some cosmetic companies which are now sourcing propylene glycol from plant-based glycerin and some of these plant-based propylene glycols are Ecocert certified.

When it comes to sourcing propylene glycol for your product formulations, it is always important to determine the chemical starting material that is used to produce propylene glycol. Do not assume that the starting material for a chemical is only derived from petroleum or natural gas.

Garde D solvent dispensing area EM requirement

It will great if you help me out with this.
Is it regulatory requirements to do settle plate and air sampling at same time? Grade D required EM by settle plate or air sampling is ok ?
Considering the Solvents have antimicrobial effects , EM required?
I have gone through usp 1115
Who guidelines.

Selection of I.V. container type for in-use compatibility

I have question, regarding the choice and justification for choice of I.V. container types for infusion solution preparation to include in in-use drug product diluent compatibility study.

The final use of our generic Drug Product is via intravenous infusion only. The drug product will be diluted with only one compatible diluent recommended in labeling for infusion. The infusion is over two hours and the prepared solution can be kept for 4 hour if not used immediately. As I found the information on internet, the most widely used container types are rigid such as glass, and flexible such as Polyvinyl chloride, Polypropylene.

My question, are there any other types of I.V. container in use at healthcare settings? If not would it be justifiable to use two as a representative of most widely used I.V container types glass and Polypropylene?

proof of contact on contact plates

When using plates for the operators fingers, there is a discussion on the need to check that the operator has made contact (looking for indent) prior to incubation. The down-side of course is the danger for contamination at the time of inspection. Inspection at the end of incubation may not always be representative as the start of incubation. Not to mention lost days waiting for organisms to grow when a contact was possibly missed. Are there any products out there that can show/confirm contact without affecting microbial recovery?

BET Limits

According to Ph. Eur. 5.1.10., the BET limit must take into consideration any theoretical bacterial endotoxin load introduced by any other components used for dilution of the product or introduced by raw materials. Therefore if any drug product is diluted before administration with any diluent for e.g. 0.9% NaCl solution or 5% glucose. Then is it required taking into consideration the bacterial endotoxin load introduced by water for injection used as excipient and the dilution solution?

Since the used drug product and diluents are already tested and released separately as per compendia monograph at their respective limits. So if they combined later, is it required to tighten the limit for drug product?

A1: It has not gone unnoticed that if all the pharmaceutical ingredients including the WFI were at their upper limit and the drug product may fail its maximum human dose calculated bacterial endotoxin specification.

In practice, it is highly unlikely that the ingredient will all be at their upper limits but you would need to calculate the possible contribution of each to the finished product.

Sterile product transportation during manufacturing through a non-classified area

I would like to ask you if it would be acceptable or if there is any regulatory issue in the following scenario for aseptic manufacturing of sterile vials:

Bulk Compounding is performed in grade C room 1 and then, sterile filtration to a tank in grade B room 1.

Then I take this closed tank with the sterile product and move it out of the manufacturing area through a non classified corridor to a new manufacturing area.

The tank is introduced in grade C room 2 and product is transferred to a grade B room 2 (no sterile filtration) for filling and capping phase.

If I validate sterile product tank transportation in a media fill batch, would it be acceptable? Do you have production processes with different phases in different areas so you have to move product thorough a non-classified area?

A1: Sure is acceptable if you integrity test the tank and conduct a media fill validation.Sanitizing the wheels and tank exterior would also be an issue.

Bulk biologic material including COVID-19 vaccines are successfully transported from biologic manufacturing facilities to fill/finish facilities all the time.

Malassezia restricta

Does anyone have experience growing Malassezia restricta (ATC #MYA-11)? I need 36 mL of a culture containing 1x108-1x109 CFU/mL. Growth media? Solid or liquid? Shaking? Incubation time? Subculture?

Any tips on enumeration? I haven't been able to see distinct colonies on agar medium, mostly smeary.

A1: According to Limon et al., 2019, Cell Host & Microbe25, 377-388March 13, 2019

"Malassezia restricta (clinical isolate MYA-4611) was grown statically for 3 days at 30°C in modified Dixon broth (mDixon) supplemented with glycerol monostearate"

A2: This is a difficult fungus to grow, esp. in plate count/quantittive culture from clinical sources. Culture methods are not that useful. Consdier https://jcm.asm.org/content/40/9/3350.short

A3: 2011 5th Edition recommends Modified Dixon Agar for culturing Malassezia spp. for 5 days at 30-35 degree C. The fungus requires long chain fatty acids in the medium to grow.I assume you would harvest the yeast-like cells.

A4: Based upon the product sheet for this strain, ATCC had recommend either ATCC Medium 269 (Modified Dixon) or ATCC Medium 2737 (Modified Leeming & Notman Agar) with an incubation temperature of 30C for growing this strain (Malassezia restricta – ATCC MYA4611).

In March 2010, there was response to a PMF post by Liz Kerrigan concerning the types of media that can be used to grow Malassezia. At the time, she had indicated that ATCC had used Pityrosporum Medium ATCC Medium 1072) at 30C to grow various Malassezia species. I have found that the archives of the PMF can be very useful at times to provide guidance.

You can try all three of the above media to grow Malassezia restricta to high levels.

Besides being a common skin organism, I suspect that you must be performing some type of anti-dandruff work because Malassezia restrict is one of the two Malassezia species that has been implicated to cause dandruff based upon gene sequencing work performed by P&G.

Virology Question

Can anyone help me on a virology test method?

Specifically on recently approved ASTM E1053.

A1: ASTM E1053 – 20 Standard Practice to Access Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces is a quantitative, carrier-based method that is used to access the virucidal activity of disinfectants. In general, it is my understanding that this method

is a surface time kill method in which an aliquot of virus suspension is dried onto the surface of a glass Petri dish. The disinfectant is applied. After the prescribed contact time had been observed, the treated virus film is scraped from the dish, collected, and neutralized. The neutralized solution is serially diluted, and aliquots of each dilution are added to four cell culture wells containing a monolayer of cells. These inoculated cell cultures are incubated and examined for cytopathic effect. A neutralization control must be performed to confirm the effectiveness of the neutralization of the disinfectant using either chemical neutralizers or a gel filtration column. The performance of a cytotoxicity control will need to be done to potentially assess the toxic effects of the chemical neutralizer itself and the neutralized disinfectant.

Because this test method is somewhat complicated, it is my recommendation that you perform this type of testing at a contract laboratory that has the necessary experience in conducting virucidal disinfectant testing if you do not have the experience required for working with viruses and cell cultures. I will admit that it takes a highly trained microbiologist or a virologist to perform this testing due to issues with aseptic technique in working with cell cultures.

A2: Thank you [name redacted], for clearly describing the method as published. I also have the copy of the method. The question I want to ask is the method does not recommend a set number of replicates of disinfectant surfaces to be tested in order to calculate TCID50. The quadruplicate plating in 4 wells is similar to our bacterial plating in duplicates or triplicates, am I correct.

When I read papers for calculating TCID 50, in veterinary sciences, they take 10 replicated of mice to infect and determine # of mice infected vs died.

But in cell cultures, how does one determine by using only on treated carrier? Can this method be carried out using only one or 2 test carriers.

Or does this test have to be done following the AOAC methods using 10 or 60 replicates of treated surfaces?

A3: I would recommend that you download a copy of the following E.P.A. document:

E.P.A. Product Performance Test Guidelines OSPP 810.2200: Disinfectants for Use on Environmental Surfaces - Guidance for Efficacy Testing.

In Tables 1 and 2 of this document, there is a listing of organisms, number of batches and replicates for each of test organism in each of the AOAC and ASTM test methods.

Related potency of antibiotics

In some cases potency of antibiotics given in units per mg and some where in microgram per mg. Both units and micrograms are same or in other words can we say 1 mcg equal to 1 unit.

For example Neomycin sulphate in USP the potency is NLT 600 microgram per mg and in European pharmacopoeia the potency is NLT 680 Units per mg. If we use USP RS potency is mentioned on vial is microgram per mg and we find result on microgram per mg. How can convert our result in units per mg as per European pharmacopoeia.

Can we apply conversion factor for conversion of Units from microgram.
OR
We shall analyze our sample neomycin sulphate from EDQM RS where potency is mentioned in Units per mg.

I am not sure, perhaps EDQM says that conversion factor is wrong practice, should use RS as per your requirement. Is it right? Please suggest.

Particulate matter in microtesting area

Please guide us that is this necessary to take particle count in microtesting area daily including laminar, surrounding rooms, as settle plate, air sampling done daily?

Depyrogenation of ovens

How do we depyrogenate oven (used in microbiology lab for depyrogenation
of glasswares for BET)?
And what should be the frequency for same?

A1: The following LAL Update may be of use to you in your depyrogenation validation endeavours.

https://www.acciusa.com/pdfs/newsletter/LAL_Vol.11No.5.pdf

A2: I think you are asking about qualification of Oven? Its depyrogenation, along with routine handling.(e.g at 200deg. C for 2 hrs. or 1hrs. at 250deg.)

Validation can be performed on yearly basis.

A3: I would have a look into USP 1228 and 1228.1 and 1228.5 (that are the general chapter regarding depyrogenation, the chapter specific for depyrogenation by heat and the one related to endotoxin indicators).

Effect of time on water testing

Due to some renovations I have to send my purified water samples to external place for the testing. What will be the effect of time on water? It will be around 18-20 hours hault, however temperature is maintained with ice packs.

A1: Which industry is it. Scientifically it should be tested before 8 hours if it is stored under room temperature/ USP controlled temperature. However you need to validate the process for recovery.

A2: Take a look at USP FAQ site for the topic "pharmaceutical water" - there are Q and A that address your general question with respect to external testing, sample storage, microbial and chemical testing, and potential challenges.
Bear in mind that these Q/A are NOT official USP text, only information to guide you. Ultimately, you need to assure the testing is representative of the water used during operation.

A3: Please. go through with *USP chapter 1231 "Water for pharmaceutical uses*"
 *section-
Microbial enumeration considerations* sample should be tested *with in 8-12hrs. *if not should store* 2-8Deg. C upto 48 hrs*, in case of off-site contract lab.

A4: Can you confirm the reference for those hold times as I was unable to find that text in USP 1231?

A5: The verbiage was added back in to the chapter in USP 41 1st Supplement.

"If it is not possible to test the sample within 2 h of collection, the sample should be held at refrigerated temperatures (2°–8°) and tested within 24 h. In situations where even 24 h is not possible (such as when using off-site contract laboratories), it is particularly important to qualify the microbiological sample hold times and storage conditions to avoid significant changes in the microbial population during sample storage.▲1S (USP41)”

A6: USP 1231 references the AWWA/APHA Standard Methods For the Examination of Water and Wastewater. 20th Edition Section 9060 B. Preservation and Storage Drinking Water for Compliance Purposes states preferably hold samples at <10 degree C during transit to the laboratory. Analyze samples on day of receipt whenever possible, refrigerate overnight if the arrival is too late for processing the same day. Do not exceed 30 hour holding time from collection for coliform analysis and 8 hours for heterotrophic plate count.

A7: USP refers back to Standard Methods for the Examination of Water and Wastewater. In that document (specifically 9060B Preservation and Storage) they state the following: USP <1231>, 8.5.1 states the following: Consideration should also be given to the timeliness of microbial testing after sample collection. The number of detectable organisms in a sample collected in a sterile, scrupulously clean sample container will usually decrease as time passes. The organisms within the sample may die or adhere to the container walls, reducing the number that can be withdrawn from the sample for testing. The opposite effect can also occur if the sample container is not scrupulously clean and contains a low concentration of nutrients that could promote microbial growth. Because the number of organisms in the water can change over time after sample collection, it is best to test the samples as soon as possible. If it is not possible to test the sample within 2 h of collection, the sample should be held at refrigerated temperatures (2°–8°) and tested within 24 h. In situations where even 24 h is not possible (such as when using off-site contract laboratories), it is particularly important to qualify the microbiological sample hold times and storage conditions to avoid significant changes in the microbial population during sample storage.▲1S (USP41)

Standard Methods for the Examination of Water and Wastewater:
9060 B. Preservation and Storage
1. Holding Time and Temperature
a. General: Start microbiological analysis of water samples as soon as possible after collection to avoid unpredictable changes in the microbial population. For most accurate results, ice samples during transport to the laboratory, if they cannot be processed within 1 h after collection. If the results may be used in legal action, employ special means (rapid transport, express mail, courier service, etc.) to deliver the samples to the laboratory within the specified time limits and maintain chain of custody. Follow the guidelines and requirements given below for specific water types.
b. Drinking water for compliance purposes: Preferably hold samples at <10°C during transit to the laboratory. Analyze samples on day of receipt whenever possible and refrigerate overnight if arrival is too late for processing on same day. Do not exceed 30 h holding time from collection to analysis for coliform bacteria. Do not exceed 8 h holding time for heterotrophic plate counts.
c. Nonpotable water for compliance purposes: Hold source water, stream pollution, recreational water, and wastewater samples below 10°C during a maximum transport time of 6 h. Refrigerate these samples upon receipt in the laboratory and process within 2 h. When transport conditions necessitate delays in delivery of samples longer than 6 h, consider using either field laboratory facilities located at the site of collection or delayed incubation procedures.
d. Other water types for noncompliance purposes: Hold samples below 10°C during transport and until time of analysis. Do not exceed 24 h holding time.

These are pretty standard sample and shipping processes.

A8: I must say that it makes no sense to qualify the storage conditions specified in a Standard Method.

A9: As EM samples are also shipped are there similar guideline hold times for EM contact and petri plates before they get incubated or is it just through each site validating their own process?

A10: Ok [name redacted], I give up. What was this in regards to?

A11: There is no issue you should perform the transportation study to evaluate the possible bioburden reduction during that period. As per USP chapter 1231 you can validate hold time of about 48 hrs.

A12: I think as per revision in USP chapter 1231, purified water can be tested for microbiological test within 24 hour of collection, which was earlier 12 hours.

A13: It is good to keep the validated procedure with approved off site contract lab for the same.

A14: I am aware and understand the reasoning for the hold time not to exceed 24 hours as per USP and the Standard Methods for the Examination of Water and Wastewater, but I have read through various responses that there may be a possibility to extend this hold time to 48 hours with validation. Is anyone familiar with this? How would it be executed? Would it be questioned as not acceptable since the industry standard is recommended not to exceed the 24 hr hold time?

A15: [name redacted] does a good job of explaining hold times for bioburden testing here. Search "Assessing_Process_Hold_Times_for_Microbial_Risks_Bioburden_and_Endotoxin"

Purified water system III validation

Can we skip the water testing on holidays during the phase III validation(There is no manufacturing going on).

A1: That depends on how you structured your Phase III validation protocol.

A2: You can mention in protocol only working days You can skip weekends.

A3: After the successful completion of Phase I and Phase II, the frequency of sampling in Phase III of a purified water system is generally reduced to the normal pattern that has been determined by the user that is based upon established procedures that have been proven in Phase I and II of the validation protocol. In Phase III, the generated purified water can be used for manufacturing. From my experience, the Phase III component of a validation program for a purified water system is generally a year in length. If it were determined by the user that the frequency of sampling during Phase III was 1, 2, or 3 times per week that is conducted during a work day of the week, I would recommend that the sampling of the purified water system not be skipped for that week if the sampling day was a holiday. Instead, I recommend that you issue a deviation to the Phase III validation protocol to justify the change in the day of sampling from the holiday to a different day of the week if the days of the week for sampling had been indicated in the validation protocol. By doing this, frequency of sampling during the week of a Phase III validation plan is still the same for that week with a holiday as with other weeks.

1:10 dilution

I am working in a tablet manufacturing facility and we are involved in testing of non-sterile drug products and substances.
While performing microbial enumeration test, as per the pharmacopoeia i am doing 1:10 dilution of the drug product or substance as below;
adding 10g of drug product/substance to 90mL of buffered sodium peptone solution pH 7.0.

I want to know, if i am doing it correctly or i need to add 10g of drug product/substance into 100mL of the diluent.

Please let me know what is the correct practice.

A1: 10 into 90 is correct.

A2: The correct is 10g with 90mL Diluent. 10g correspond to 10mL. 1g=1mL

A3: Technically it would be best to add 10 g of sample to an amount of diluent to obtain 100 mL final volume. However, the standard in the industry is as you described, 10 g of sample to 90 mL diluent.

A4: The USP General Notices section 6.50.20 explains the difference between expressions such as "1 in 10" and "1:10." These are not exactly equivalent solutions. The use of the colon in describing solution

components is best reserved for mixing liquids. USP <61> indicates that sample preparations will usually be 1 in 10 unless further dilution is needed to neutralize antimicrobial properties of the sample material.

1 in 10 would be 10mL (or 10g) into 90mL of your diluent
1:10 would be 10mL mixed with 100mL of diluent

In short, I believe you are correct in mixing 10g into 90mL.

A5: 10g in to 90ml would be correct when interpreting 1:10 (or 1/10 or 1 in 10) for a dilution of product for microbial testing.
This approach and interpretation is a standard practice within the field of biology.

A6: 10 g of sample in 90 ml of diluent and use 9 ml of that diluted product which corresponds to 1 g of the product for the test. Am i right?

A7: 10 + 90 = final volume 100 ml - dilution 1:10 to have 1 g of product you have to take 10 ml.

A8: You would use 10mL of the diluted product to correspond to 1g of product.

10g product
90mL diluent
=~100mL "solution"

10mL product / total volume of 100mL = 1mL product / 10mL solution

Paper Carrier Biological Indicators Population Verification

I am wondering how companies are performing chapter USP <55> for paper carriers (G. stearothermophilus).
Are you using a blender, glass beads, vortex, sonication, etc?
Are you using 4 strips as the USP suggests?
The paper carriers are so hard to emulsify and ensure that all the spores have been released.
We get much better recovery when doing a population check for ampule solutions. Looking for some pointers.

A1: Alison - We put the strips into a glass test tube and add glass beads to the tube. We then vortex until the strips are completely macerated. This takes between 5 and 10 minutes. The beads make all the difference. We have tried blending but vortexing works much better.

We have tested 4 individual strips and 4 strips pooled together. Both ways work. If you have 4 pooled you may need to vortex a bit longer.

A2: The blender is the best option in my opinion. But you need small volume of blender like 350 ml. For example when you perform with 1lt it does not work. Because the strip stick the walls or cap of blender. I tried glass beads and sonication but could not have recovery. Also suggest you to want method from your supplier. I used 3m s method.

A3: Check with your BI manufacturer. One of the issues that may come up is that if your count is unable to verify the manufacturer's cert, they will ask you how you performed your population verification. If you used one method and they used another, it could not be considered equivalent.

A4: The Waring Laboratory blenders have mini-sample container accessories and fit to the blender base with volumes 12 to 37 mL, 37 to 110 mL or 50 to 250 mL capacities.

By the way the band leader Fred Waring developed the blender to mix his cocktails after a performance.

A5: I recommend that you contact the BI manufacturer and follow the method that they recommend for BI enumeration and used to determine the listed, cert population value. I've seen slight deviations from the manufacturer's method, sometimes lead to substantial differences in recovery.

ISO 11138-1 is a reference method that allows for greater flexibility, in BI maceration/homogenization method.

A6: Solving paper carriers are so hard in 100ml sterilized Purified Water so that I had been trying firstly mixed 3 paper carriers in the 10 ml test tube by using a few sterile disposible inoculation loop and then completing to 100ml volume. In addition I think you may recovery much more from indicators whether you exposure the stock solution in ultrasonic bath for 15 minutes.

Low Endotoxin (LPS) Recovery (LER, LLR)

I have been googling LER or LLR and Kolliphor (a poloxamer, classified as a nonionic emulsifier) and have found nothing. However, I have found plenty about other non-ionic surfactants causing LER. Does anyone have experience with Kolliphor and whether it is or is not an issue with LER? Or does anyone have the chemistry "chops" to say why it would or would not be?

A1: We don't have specific experience with Kolliphor. Given that LER is a dynamic relationship between the chelator, non-ionic surfactant and the tested substance, it is best to perform the hold time studies following the guidelines in PDA Technical Report #82.

A2: While chemically similar to PEG, also found in reference standard endotoxin and accordingly unlikely leading to LER, poloxamer shows a tendency to self-aggregate in aqueous solutions. Given its amphiphilic nature, I would not be surprised, if it caused LER at some concentration. Moreover, active pharmaceutical ingredient(s), other excipients and the storage container/vial can have a critical impact on endotoxin intermolecular structure and thus LER, potentially synergistically with poloxamer. A hold-time study with (ideally reference) standard endotoxin spiked into undiluted product and, as control, water will bring clarity.

Generally, I have seen intrinsic endotoxin ("autochthonous"/"environmental" ...), endotoxin-containing bacterial culture supernatants ("naturally occurring endotoxins" NOE ... how very natural) and purified lipopolysaccharides exhibit low recovery with valid positive product controls after exposure to certain substances. Differences between these purity levels, if taken from the same original strain, have not been demonstrated. Moreover, lipopolysaccharides themselves are the TLR4/MD2 ligands and, thus, the effective endotoxin/pyrogen.

I do not mean to be offensive at all, but based on this, I would argue that all purity levels of lipopolysaccharides are potential subjects to LER. Accordingly, LLR would primarily be meant to defend the ostensible infallibility of the bacterial endotoxins test by some of its reagents' manufacturer(s).

A3: Kolliphor® EL (formerly Cremophor-EL), like Tween-80 (T80) and Tween-20 (T20) is a nonionic surface-active compound made up of hydrophilic and hydrophobic moieties (amphoteric). In aqueous solutions, molecules will self-aggregate above the critical micellar concentration (cmc ~0.02% w/w) to form colloidal particles, spherical micelles with a lipophilic interior. Masking by micelle partitioning seems very likely above the Kolliphor CMC.

A4: Curious … is that cmc 0.02% universal or specific to Kolliphor?

if the %Kolliphor is 0.001% … would that mean masking is unlikely, or would a hold study still be I the cards?

A5: The CMC estimate I gave came from a reference in the literature: European Journal of Pharmaceutical Sciences 111 (2018) 96–103

Critical micelle concentration is unique to each compound's structure, and also has a dependence on additional factors such as temperature, and other dissolved species(other surface-active substances and especially for ionic surfactants the concentration of any electrolytes).

If the %Kolliphor was 0.001% by weight, and the CMC is ~ 0.02% (wt), it seems very unlikely that is masking due to Kolliphor.

A6: I should have been more careful in my last response. With the low concentration of surfactant, masking by entrainment in a micell is very unlikely, but that hasn't been the proposed mechanism of LER. I believe there hasn't been a clear mechanistic explanation of LER, just the association of surfactants with chelators and some dependence on the endotoxin source (purified vs NO).

EM Equipment Qualification

I have two new SAS Viable Air samplers and one new non-viable particle counter I need to qualify for our existing validated sample methods. Sample size of 200L will be observed but differences in flow rate and microbial recovery in SAS versus RCS seem to be the major differences.

Existing Equipment -

- Particulate - Lighthouse Solair 1cf/m flow rate (3.5 min sample, 200L)
- Viable - Biotest RCS 100LPM (2 min sample)

New Equipment -
- Particulate - Lighthouse ApexZ50 100LPM flow rate
- Viable - Bioscience international 100LPM flow rate

My industry is non-sterile manufacturing of clinical IVD's (low risk environments) and I'm struggling to find guidance on how to execute a side by side comparison to our existing equipment to the new. models that is commensurate to my industry in not having to do some extensive comparison.

Does anyone have any suggestions? All i should need to display is equal or better detection in both systems but duration and statistically displaying this seems to be the issue.

A1: Qualifying viable samplers side by side isn't necessary because air isn't homogenous, impaction is different and it's impossible to take a few data points and make any conclusions. The FDA doesn't have any expectations of a firm to perform side by side testing. If you did want to do this, I would suggest performing a longer study over the course of a month and try to statistically compare counts. Depending on your counts, you may see equivalence, you may not, particularly on your very short sampling times. Each viable sampler manufacturer should have performed validation studies internally showing physical and biological efficiencies according to ISO 14698 (now EN 17141) and Biotest should be able to provide some validation studies to help your situation but if they don't have that data, you may want to inquire further.

A2: For viables, I find myself in a comparable situation in trying to compare two different viable impaction samplers with different agar impaction surface areas as well as different numbers of pores in the sampling head. When counts are low, the comparison seems straight-forward, but in some non-sterile environments, as counts increase, the comparison becomes less accurate. This has been described in J.M. Macher's paper 'Positive-hole correction of multiple jet impactors for collecting viable microorganisms'. With an equation from her paper, a table can be made (or is often provided with the sampler) based on the number of sample head pores to give a theoretical count when higher counts are expected.

We went from the Sartorius MD8 sampler (410 pore head, 100 L sample volume) to the Microbiologique sampler (161 pore head, 100 L sample volume).

I also could not find good literature on guidance for comparison so we may have to get creative.

environmental monitoring

I am hoping someone, anyone, can help me with guidance on environmental monitoring. We currently monitor air and water in our non-sterile pharmaceutical manufacturing plant based on our trending data. However, we are going to begin touch plates and swabbing, and I am a little overwhelmed. I have quantified swabs before by swabbing and immersing the swab in broth, vortexing, and then plating. However, how would you quantify touch plates such as RODAC plates? What if an organism spreads all over the plate and you cannot get an accurate count. Would these be used more as an indication of the environment rather than providing passing or failing results? For a non-sterile facility, is it best to perform swabbing and touch plates on high touch areas from people and not product. If I were to swab a tank, they have to re-clean the entire tank which takes time. Any suggestions are greatly appreciated!

A1: Environmental monitoring is generally performed in the background environment. Sampling of equipment internals may be done to support cleaning validation efforts or as part of your routine cleaning verification program. Though you can certainly swab the background environment, most EM programs focus on nonviable particulate monitoring, active viable air monitoring, and touch plates. In manufacturing steps of high risk to your product, you may also wish to expose settling plates (ex: a compounding step in a biosafety cabinet). Personnel monitoring is also a common component of EM programs.

Your procedure should dictate how you handle spreaders. You may count it as a TNTC and follow your action limit response (typically a clean of the area and then re-sampling) or you can indicate it is a spreader and unable to obtain a valid count. This should also lead to a re-sampling event to obtain a valid result.

Environmental monitoring of your background environment is typically not used alone as release criteria. That is, a "failing" EM result (action limit excursion) does not necessarily mean you have to hold/scrap your batch. There are, of course, a lot of nuances, but it really depends on how you build your program and which samples you consider "environmental monitoring" vs something like in-process monitoring of your process.

I'm not sure which regulatory authority you fall under, but PDA tech report 13 covers environmental monitoring, and EU GMP Annex 1 does as well. ISO 14644-1 helps guide non-viable particulate monitoring.

A2: There are no standards in the ISO charts for gloves in a non-sterile environment so you'd sort of be starting from scratch as to how to establish your alert and action levels. Environmental monitoring is just that...monitoring so it's not a "release test"...but if you continually have very high counts that exceed your alert and action levels, that will draw some attention. Also, there are many studies that you can find that talk about recoveries of organisms on contact plates...and those numbers vary. You'll also need to take into account the sanitizer/disinfectant you're using and whether or not your media can neutralize it. If you'd like to contact me directly, you can reach me at [log in to unmask] for documentation on recoveries and neutralization.

A3: The use of swabs and contact plates are considered Class O standard methods, e.g., Standard Methods For the Examination of Dairy Products and have been subject to a thorough evaluation and are widely used so may be considered grandfathered.

The surface contracted with a plate is wiped down with an alcohol wipe to remove media residuals while swabs are not expected to leave residues.

As discussed in USP <11115) Bioburden Control in the Production of Non-sterile Products do not overdo the EM in non-sterile manufacturing areas.

A4: Sorry for being late to the party. I agree with [names redacted]. I haven't seen a company use touch plates for personnel in non-sterile manufacturing yet, but there is always a first time.

The touch plates will be dirty considering you will be monitoring the dirty gloved fingers of operators in a non-sterile environment. I would recommend doing a feasibility study for a period of time. Monitor the operator's gloved hands at several intervals and time points over a period of time (e.g. month). And then review the data to determine realistic action limits that are fit for your purpose.

A5: In general, the performance of doing touch plates in a non-sterile environment is never performed. Swabbing of equipment surfaces is perform to either validate the sanitization processes or to monitor the compliance with a validated sanitization procedure. RODAC plates may be used to take samples of flat surfaces of the building such as walls and floors after sanitization. Air samples are usually taken of the manufacturing environment by using either gravity settling plates or a mechanical air sampler. Touch plates using RODAC platted are normally performed for workers who normally work in a Class 100 environment and wear sterile PPE and uniforms. From my experience, I have never seen anyone to perform particulate (non-viable) counts in a non-sterile manufacturing environment, but there can always be someone that does it because they have a classified manufacturing environment. For non-sterile manufacturing, the manufacturing environment is generally never classified to meet even a Class D or Class 100,000 environment. Why conduct particulate counts in the first place? Workers do not wear sterile uniforms. It is hard for workers to maintain clean uniforms in this type of environment depending the work area such as compounding. I have seen many times workers wear non-sterile gloves, hair nets, beard covers and disposable arm sleeves to cover bare arms if they are wearing a short sleeve shirt or blouse of a uniform to prevent contamination of a non-sterile finished product. By the way, I have seen companies in which there are different color worker uniforms for the identification of job function in a non-sterile manufacturing environment. I have even seen some companies to have their workers wear a non-sterile surgical mask over their face during the manufacturing of non-sterile finished products. During a workday, it is very normal for workers to change their non-sterile gloves immediately if they become soiled and to use alcohol hand sanitizers several times a day if their gloves are not soiled. I will be honest that I do not see the purpose of conducting touch plates of workers in a non-sterile manufacturing environment because you are allowed to have a low microbial count in a non-sterile product unlike sterile products. I suspect that workers in a non-sterile manufacturing environment will normally have higher counts than workers in a Class 100 room if samples were ever taken by using a touch plate.

A6: We won't be doing touch plates on personnel. There are too many variables that we would have to consider. We were thinking of doing touch plates and swabbing on surfaces.

A7: Only because a dozen opinions are never enough when it comes to EM!

[name redacted], let's try make this simple (and I apologize ahead of time if I am unable to do so). As to your original question:

Any EM activity that occurs in unclassified/non-sterile operations are risk-based (for information, please have a read of <USP 1115>) with little, if none, regulatory guidance given. I'll assume by touch plate you are referring to surface monitoring via contact plate. My question would be are you then targeting facility surfaces (e.g. walls, windows, floors) with your contact/RODAC plates and swabs or equipment surfaces? You can quantify swabs and contact plates as they are designed as such, assuming you are using a sterile diluent with your swabs. One method is to vortex the swab head (aseptically snapped off from the stalk) in the diluent and membrane filter for a count (whereas using TSB as your swab liquid is qualitative only and should be reserved for use in ISO 5 environments, which is not applicable for you). If you are swabbing a tank and exceeding your alert level or action limit, then you would clean the tank as it would be an indication of poor cleaning/disinfection (unless you have personnel in your tanks, in which case, STOP doing that).

For your direct quantification of contact plates, you just count them. That is all (I wish I could offer more than that). If there are spreaders, then you would want to incorporate an interim read on your plates during your incubation period. Spreaders, in my opinion, are always a qualitative excursion and should be investigated as such.

Both methods have limitations on recovery and you can conduct a study demonstrating your recovery percentages with your media or swabs, in your facility, on your representative surfaces by conducting a coupon evaluation with known counts of appropriate microorganisms.

From an indication standpoint, all EM is in fact, an indication of the environment! The passing/failing is somewhat misleading based on the capabilities and data generated, which is why alert levels and action limits are established. You will have to look at each sampling location and determine what your course of action would be for alert/action excursions. EM data should be utilized to determine state of control and when you are drifting from that happy state of being. Therefore action limit (or multiple alert level) excursions, which would prompt an investigation and CAPA, can be directed towards cleaning & disinfection improvement, facility improvements, personnel behavior improvement or process improvement. The choice is yours and is based on the outcome of your investigation.

Ultimately, I will answer your question with "it depends".

A8: We also produce non-sterile solid dosage forms and implemented all of the mentioned (exception: settle plates) for our non-classified production area.

As we now changed to GMP class D (by EU-GMP-Guideline), I may give you a short overview on our previously used system:

1) We called it class E in the style of GMP class A-D (suggested by our authority).
2) We defined limits based on historical data, balanced by GMP class D limits and some literature.
3) Sampling points were defined risk based.
4) We used contact plates for non product contacting surfaces (floors, walls, etc.). -> monitor the cleaning and disinfection process within the rooms
5) We used contact plates for product contacting surfaces (inner side of the systems, etc.). -> monitor the cleaning and disinfection process for the systems (cleaning validation)
6) If not reachable, we also used swabs for testing product contacting surfaces (tubes, etc.). -> monitor the cleaning and disinfection process for the systems (cleaning validation)
7) We measured the viable air particles by an active air sampler. -> monitor the HVAC-systems and personnel activity in the production area
8) We measured the non-viable air particles by a particle counter. -> monitor the HVAC-systems and personnel activity in the production area
9) We used contact plates for personal samples (hands & gowning). -> monitor the personnel hygiene (Did they wash, gown and disinfect properly ?)
10) We take samples from utilities (soap, creme, detergent) within the production area. -> hand dispensers cleaned and disinfected properly ?
11) We take samples from cleaning agents and detergents used in the cleaning areas and for cleaning and disinfection of the rooms. -> removal tools cleaned properly ?

polyethylene sterilization validation in BFS machine

For Blow Fill Seal machine do you perform the sterilization validation for polyethylene (packaging) material? If you do how do you perform?

A1: What kind of validation do you mean? Asking for polymer? For polymer its possible to perform bioburden validation. Bu the sterility of polymer you can see during media fill.

A2: Yes, it is about polymer. We were asked about polymer sterilization validation. We found some documents, they contaminate the polymers and then fill the vials with TSB and investigate if there is growth or not.

A3: Next-generation BFS machine, you can only see in media fill validation.

Acceptable limits on duplicate plate counts

I would like to know when performing "duplicate" plate counts i.e. TAMC/TYMC what would be considered acceptable results for low recovery counts? For example: let's consider a situation where one plate had 10 colonies and the other had zero (0) colonies, or even say 4 on one plate and zero (0) on the other. Should these type of results be invalidated and require a retest to ensure accuracy? Or would such a variance in duplicate plate counts be acceptable (taking into consideration the stochasticity of microbes) and the final result would be the higher count e.g. 10 colonies x the dilution factor? If acceptable, where do we draw the line about limits on replicate low recovery plate counts?

I am operating to the BP Appendix XVI B Microbiological Examination of Non-Sterile Products.

A1: From the FDA BAM:
Aerobic Plate Count

Guidelines for calculating and reporting APCs in uncommon cases

Official Methods of Analysis (3) does not provide guidelines for counting and reporting plate counts, whereas *Standard Methods for the Examination* *of Dairy Products*, 16th ed. (2) presents detailed guidelines; for uniformity, therefore, use APHA guidelines as modified (6,8). Report all aerobic plate counts (2) computed from duplicate plates. For milk samples, report all aerobic plate (2) counts computed from duplicate plates containing less than 25 colonies as less than 25 estimated count. Report all aerobic plate counts (2) computed from duplicate plates containing more than 250 colonies as estimated counts. Counts outside the normal 25-250 range may give erroneous indications of the actual bacterial composition of the sample. Dilution factors may exaggerate low counts (less than 25), and crowded plates (greater than 250) may be difficult to count or may inhibit the growth of some bacteria, resulting in a low count. Report counts less than 25 or more than 250 colonies as estimated aerobic plate counts (EAPC).

Covid-19 mRNA Vaccines

Generally, single dose vaccines do not contain preservatives. However, preservatives are commonly used in multi-dose vaccines. From what I can determine, the most common preservatives that are used in vaccines are thimerosal, benzethonium chloride, phenol and 2-phenoxyethanol. Based upon information on CDC and FDA websites, thimerosal is not used in vaccines for children. However, thimerosal is used as a preservative at a 0.001 to 0.01% concentration in tetanus and flu vaccines, benzethonium chloride is used in anthrax vaccines, phenol at 0.25% concentration is used in vaccines to prevent pneumonia, smallpox and typhoid fever, and 2-Phenoxyethanol at a 0.5% concentration is used in polio vaccines.

In looking at the ingredients that are present in each of the mRNA vaccines that contain 5 doses for patients, there are no preservatives present in either formulation. I understand that the low storage temperature is used to prevent the degradation of the mRNA. I'm suspecting that because the frozen vial is used that day to provide 5 inoculations that there is no need or the risk is considered to be low to include a preservative in these multi-dose vials. However, I'm not completely sold on this hypothesis as to why a preservative is not included as an ingredient in each of the formulations.

However, I do see that in some children vaccines have high concentrations of either Sorbitol or Sucrose are being used in comparison to the other ingredients. It is well known that high concentrations of sorbitol and sugar such as dextrose and sucrose have been used in the past to preserve product formulations due to their humectant activity to lower a formulations water activity. In each of the Covid-19 mRNA vaccines, I see that Sucrose is present as an ingredient (see FDA website for the composition of these vaccines) to help the molecules maintain their shape during freezing. Besides maintaining the shape of the mRNA molecule during freezing, I'm wondering whether this ingredient of sucrose is also contributing to the preservative adequacy of these mRNA vaccine formulations.

A1: Although it is a multiple dose sterile product, the Pfizer dilution and storage time is limited to 6 hours so not having a preservative system is justified.

A2: No preservatives is what I've read also. Also read Moderna is thawed and administered, and Pfizer is thawed, diluted with sterile physiologic saline (0.45 ml vaccine coincentrate with 1.8 ml saline) and administered. https://www.fda.gov/media/144413/download and https://www.modernatx.com/covid19vaccine-eua/providers/dosing-administration
Understand covid genome codes for 29 proteins. With mRNA in vaccine(s) - does anyone know if a general purified mRNA fraction or one for a specific protein?

A3: Without the presence of preservative in the multi-dose vaccine, I would hope that the 6 hour storage time after being unfrozen is based on some type of challenge test data and not on a theoretical exercise. In Microbiology, I have found that decisions based upon only on theory gets you into trouble. At one time, I believe that the Ph EU 5.1.3 Efficacy of Antimicrobial Preservation, there was a 6-hour sampling point in which the A criteria for parenteral preparations had a 2-log reduction.

A4: I have no knowledge what the sponsor Pfizer supplied in the regulatory submission to justify the fill volume, freezing and thawing conditions, dilution and holding time. Extending the number of doses obtained from a vial may promote coring and would incrementally increase the risk of microbial contamination. Typically challenge studies would be conducted to justify the hold time or a short default time may be requested when the product formulation is less likely to support microbial growth.

USP 61,62 Sample preparation

USP 61,62: "Prepare a sample using a 1 in 10 dilution of the product".

For example,
I dissolve 10 g sample in 100 ml diluent
But I dilute 10 ml sample in 90 ml diluent.
Are you doing in the same way?

Change EDI in water system

In out company we are about to replace old EDI with new one with the same specification from the same manufacture, May I ask , what is the required validation steps ?

B. cepacia testing as per Ph. Eur

As per official chapter <60> of USP, the new requirements of products prone to this objectionable Microorganisms has a defined method. My question to the forum is that, is there a requirement defined in European union or in UK. I.e. for Eu. Phr. If yes, where is the reference for this as current European Pharmacopeia does not define this.

A1: Currently it’s only a USP requirement.

A2: As far as I am aware it is currently only a United States requirement and has not yet been harmonized with the EP. We are not performing the testing on any of our products being sold in the EU.

A3: USP <60> is a general test method that describes the test and recommends its use for screening aqueous non-sterile drug products for B. cepacia complex.

Currently, the absence of Bcc is not a requirement in USP <1111> or is found in any USP monograph.

Drug manufacturers may unilaterally use the test for their products or at the request of a regulatory agency.

Like the <62> tests for specific microorganisms, <60> is highly specific for the target organisms and is not suitable for the general screening for objectionable microorganisms.

We will have to wait and see what the Ph. Eur. will do.

A4: No, there is no Burkholderia chapter in EP but I hear it's being considered.

Regarding growth promotion test

When growth promotion test of in house prepared media perform either after 48 hrs of preincubation of media or same day i.e media was sterilize.
We have performed growth promotion test of our media and after gpt, we store the media in incubator at 20- 25degree for 20 days. If we use this media for product testing after 10 days then again requirement of gpt of media or use media plate parallel product testing as a positive control just for verification.
What is difference between gpt and positive control?

A1:

1. I do not see the need to wait. The media purity check should be separate from the gpt.
2. I would recommend storing media at 2-8C rather than 20-25C. If you are placing it into the 20-25C for a purity check of the media, is this in line with how you will be using the media for testing (20-25C incubation for 20 days)? Alternatively from needing a positive control or repeat gpt, you could perform post expiration gpt on the media. This demonstrates that the media was good through the expiration date. After enough data, you could pull back from doing each batch to once every quarter, 6 months, year, etc depending on the frequency of batches.
3. A positive control is just demonstrating that the media can still support growth. You aren't looking for percent recovery against a previously released batch; just looking to ensure growth is obtained by the media that was treated under the same conditions of your test.

Endotoxins testing of compressed gases

Recently gone through the ISO-8573 guidance {ISO 8573-7:2003(en) Compressed air — Part 7: Test method for viable microbiological contaminant content/Annex C sampling of endotoxins} and seen about testing of compressed gases for endotoxins.

I’m wondering how the endotoxin testing is done for compressed gases. What are you practicing . If yes what is the sampling method and what is allowable limits?

A1: Endotoxins content estimation in compressed air, it is not at a regulatory requirements, we have to apply science, here ,we have to keep in mind, in pharmaceuticals compressed air line filters and point of usage filters are in place for filtered compressed air.

Possibility of endotoxin contamination through filtered compressed air is not possible.

Note: The proposal endotoxin testing is not required.

A2: Bacterial endotoxin can pass through sterilizing-grade filters.

Larger pharmaceutical plants have pharmair systems to deliver oil-free, dry compressed air. The critical issue generating dry compressed air and having no condensation in the distribution lines.

Regarding rest condition in microbiology lab

We have perform sterility test in control environment (classified area). We need non viable particle count test in classified area. What is the rest condition and operation condition in microbiology lab where we perform sterility test?

A1: It may help you if you think of the testing as your production activity in the laboratory. Rest condition is with no testing occurring and operation is while sterility testing is ongoing. Specifics of how to conduct the particle testing will depend on the laboratory set up.

A2: Please suggest In rest condition, HVAC and LAF is ON condition or OFF condition.

A3: CONDITIONS AT REST: it means that the room is fully functional at the plant level, but that you are not working on the premises, i.e. there is no flow of material and personnel, but you must test the actual operating conditions of the clean room

A4: HVAC and LAF should be ON.

Method validation of sterile products

Please suggest method validation of sterile product should be perform in controlled area (sterility lab) or in uncontrolled area. Because we can not carry microorganism in sterility lab. Where we perform method validation.

A1: Many firms have a separate hood and sterility pump for method suitability in a different area of the QC Micro lab.

A2: We used to perform the method validation is a BSC in the laboratory. It did not enter the clean area.

A3: I would recommend that the sterility test suitability study not be done in the sterility laboratory. Remember that you are handling live organisms in this study and microbes have an uncanny ability to "get loose." Do the suitability in a BSC separate from your sterility test areas.

Disinfectant validation

I have a question regarding the validation (according to USP1072 / EN13697) of disinfectants for use in cleanrooms of pharmaceutical production.

Various regulations (e.g. PIC / S PI007-6, FDA Aseptic Guide, AMWHV and Annex1) require that the disinfectants used in (aseptic) manufacturing facilities must be validated with regard to their suitability and effectiveness for the intended location by use of representative surfaces and in-house germs.

When checking our production areas and surfaces for validation, we had a fundamental question about the interpretation of this requirement. In your opinion, how precise is the definition of the areas for which a validation is necessary according to guidelines?

For e.g. all clean room classes in a manufacturing environment, i.e. clean room classes A, B, C and D?
Or only for the area of aseptic production (work with open product, surfaces with product contact), i.e. clean room class A and B.
Or also preparation zones in area C?
What is the situation apart from aseptic production in the case of maybe active ingredient production?

How do you handle it or how do you define the general requirement for validation of disinfectants? Or are there guidelines that define this more precisely?

A1: This generally means what type of material...stainless steel, vinyl, PVC, etc. There is a user safety factor and environment safety factor as well that must be taken into consideration....ie toxicity, flammability, corrosion.

A2: I recommend you evaluate all non-product contact surface material of construction (MOC) in all your classified rooms. For example: ceilings, walls, floors, benchtops/counters, etc. This would mean Grades A-D (CNC not required).

Room cleaning is a critical component of your contamination control strategy, so you need the assurance that your disinfectants are efficacious against all surfaces they contact in all of your cleanrooms. Your less controlled rooms are essential for the step-wise cleanliness and protection they provide for your most critical zones.

A3: You address the use of disinfectants in all areas by identifying the materials of construction and use coupons of those materials for the surface challenge using your environmental isolates.
For in situ studies you would want to demonstrate reduction of bioburden in an area where you can depend on recoveries typically taking samples from an area in use and then after disinfection is completed. You would not need to do in each grade provided the surfaces are similar.

A4: As you state disinfectants used in (aseptic) manufacturing facilities must be validated with regard to their suitability and effectiveness for the intended location by use of representative surfaces and in-house germs.

Laboratory studies would be independent of the room cleanliness classification.

A5: An approach that I used that was accepted and appreciated by several regulatory bodies is as followed:

 * Make an inventory of the surface materials that are subject to disinfection in you manufacturing facility and assess these based on prevalence and criticality (e.g. direct, indirect, no product contact / present in what room classification). Based on your criticality / risk assessment make a selection of the surfaces to include in the challenge study.
 * Based on environmental monitoring data trends identify the micro-organisms that are most predominant in the facility. Make a top-3 or top-5 from each type (Gram positive cocci, Gram positive rod, spore forming bacterium, Gram negative rod and a fungi) to prevent you will end up with only Gram positive cocci like Micrococcus or Staphylococcus species. Based on your results, select at least one strain from each type and include this in you microbial challenge panel.
 * Some companies include the ATCC panel as well in their coupon study.
 * Create a study protocol describing the method including contact time, neutralisation method (including demonstration it is effective) and acceptance criteria, execute & report.

This is a very high-level description but hopefully it gives you more guidance.

A6: I typically recommend taking a look at your "worst case" environmental isolates in all of your cleanrooms (Grades A,B, C, and D) when choosing isolates for your coupon studies. I would also recommend conducting a risk assessment for your coupon studies on the surfaces in the cleanrooms and you can use criteria such as surface roughness, proximity to production, and how prevalent the surface is in your cleanroom operation.

I think another very good reference is PDA's Technical Report #70 on cleaning and disinfection.

A7: I would like to add one additional question:

How do you think about an isolator of an aseptic production? In this case surfaces in the isolator are cleaned with a disinfectant in preparation phase. After that, a VHP decontamination is carried out. The

production department has now argued that the surfaces do not have to be taken into account for the disinfectant validation due to the subsequent VHP decontamination.

How would you think about that? Do you include isolator surfaces from the isolator in the validation (stainless steel, format parts,...), or due you also argue with regard to VHP decontamination?

A8: What is the purpose of disinfecting isolator surfaces prior to VHP decontamination? Cleaning is one thing but to disinfect is counterproductive to using a validated VHP exposure cycle.

You may want to consider eliminating this extra step and performing some efficacy studies using VHP on relevant materials of construction normally found in your isolator. Alternatively, the isolator supplier may have plenty of data for you to reference, allowing you to justify not performing these studies and just relying on your standard biological challenges. A risk assessment should allow you to make the decision on what should be performed in-house.

A9: I would not recommend PDA TR#70 as they recommend an acceptance criterion of 1 log reduction which makes the judgement of the task force highly suspect.

A10: Re: isolator cleaning, I typically see this treated more as product-contact cleaning (i.e.: in scope of your cleaning validation program), which would require either validation or ongoing verification. Isolator monitoring is performed in terms of batch manufacture and I like to think of it separately from room EM.

Room EM: monitors room cleaning, gowning/hygiene, aseptic behaviors, material/personnel/waste transfer etc. Is performed independently of product manufacture.

Isolator EM: monitors condition of your isolator during batch manufacture. Usually consists of in-process EM (ex: settle plates) and post-batch EM (ex: touch plates on isolator gloves). Is performed in tandem with batch manufacture but independently of room cleaning/room EM. Passing in-process and post-batch EM should be release criteria on a per-batch basis.

A11: I agree with [name redacted].

It may be useful to use a sporicidal to wipe down media, samples and equipment entering a transfer isolator especial surface contacting the shelving.

A12: If transfer isolators are used, I would expect these have gone through a similar VHP validation protocol as the main isolator, eliminating the need to disinfect containers going into the transfer isolator and ultimately being introduced into the main isolator. This would apply to sterility and manufacturing isolators.

Challenging Bcc for AET

Now that USP <60> has become effective and Bcc is being included as part of the MLT program, I would like to get some opinions on challenging the Bcc organisms during AET.

Some companies are adding these organisms as needed, and some have added them as part of the normal AET program. I would like to get a better sense of where the industry is going in regards to this matter.

A1: USP <51> states: "Challenge organisms are generally based on likely contaminants to a drug product while considering its physical attributes, formulation, and intended use. The standard battery of challenge organisms described in this test need not prevent the inclusion of other species of microorganisms if deemed useful to measure the biological activity of the preservative system for a specific product. These supplemental challenge organisms are not within the scope of this chapter, but may be added in addition to the described test organisms".

For aqueous non-oral non-sterile drug products, I would consider adding B. cepacia to the challenge organisms in USP <51>.

A2: The usage of Burkholderia cepacia is not a USP 51 required test organism. It is my understanding that the PCPC challenge test methods for cosmetics include Burkholderia cepacia as an option. From my perspective, it is worthwhile to include Burkholderia cepacia ATCC 25416 as part of the challenge test organism panel because of it ability to become resistant to the antimicrobial activity of preservatives. For more information on this, please see my recent book chapter on “Preservative Resistance” in the recent book publication called Cosmetic Microbiology , A Practical Approach edited by Phil Geis. In the past, I had recommended on this forum that Burkholderia cepacia should be added as a challenge test organism in the USP 51 AET. I have heard that some companies are now including Burkholderia cepacia ATCC 25416 as one of their challenge test organisms.

A3: Per USP <51> other organisms may be used to supplement the compendial organisms if "deemed useful to measure the biological activity of the preservative system for a specific product." You can interpret that to mean if Bcc is a likely or potential contaminant of your product, then you would gain useful information regarding the effectiveness of the antimicrobial system by including Bcc as a test organism.

A4: If I were running a company with a non-sterile, aqueous product, I'd definitely be adding a BCC organism to my AET if it's not already in. You'd need to use an EM bug anyway, why not BCC? Even though the source is water, that organism has too much significance with this product classification.

A5: Some of my clients have already included strains of BCC in their AET program, including isolates that have been recovered in product, process samples and/or the facility.

A risk assessment based on the types of non-sterile products you have, intended patient population, route of administration (especially for aqueous oral dosage forms) and prior AET and/or bioburden data can help to determine what you should consider.

A6: USP <51> has no requirement to add an EM isolate.

A7: What are your thoughts on testing Bcc for oral products?

A8: I disagree with [name redacted]. For a standardized test method for product development and stability testing it is better to use a type culture than plant isolates.

A9: The answer is YES. I would refer to the Pharmtech incident in which BCC-contaminated oral docusate sodium products caused injury and death to patients.

https://www.cdc.gov/hai/outbreaks/b-cepacia/index.html

https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-2017-burkholderia-cepacia-contamination

A10: We can disagree; however, I offer the following points to consider:

USP 51 does not identify a specific BCC type culture to use during the AET; therefore, the use of BCC is not standardized.
When considering strains to use during AET, one should consider not only “standardized” strains as described in USP 60, but also strains that have been recovered from process and/or product. I have experienced wild type strains that were more resistant to preservatives that the ATCC strains identified in USP 60. For this reason, many of my clients have performed AET using ATCC and wild type strains.
Some companies have experienced BCC contamination post launch of their products (e.g., when processes or water systems have become contaminated), and these included products that were never initially assessed for efficacy against BCC. For these companies, they have elected to perform AET using ATCC and the wild type strains to assist in their risk assessments.

A11: [name redacted] I appreciate your position.

My experience with AET was the Bc type strain selected was more resistant than the Pa, so the preservative system we developed for new products would be more robust. Although Bcc has been involved in product-related outbreaks, we do not test products with multiple strains of the other challenge organisms. Also product and environmental isolates reportedly lose their resistance to preservatives when they become laboratory cultures.

For consistency a company requires a standardized test.

A12: I'm in agreement with your statement about using the type and wild-type strains of Bcc for challenge testing. I have often found that wild-type strains of Bcc are more resistant to preservatives than Burkholderia cepacia ATCC 25416. From my perspective, it is better to use the Bcc wild-type strains with the type strain (ATCC 25416) during product development phase of a product testing in which initial and artificial aged product samples are challenge tested for preservative adequacy. For real-time stability testing of marketed product batches, I would prefer the usage of including only the Bcc type strain (ATCC 25416) due to issues for culture maintenance of the wild-type strains. In general, preservative resistant strains are only resistant to the antimicrobial activity of one preservative such as Benzyl alcohol, parabens, MCIT/MIT,Sodium benzoate, DMDM hydantoin and others. It depends upon the selective pressure in the facility that had generated the Bcc preservative resistant isolate. It is very impossible to find a Bcc preservative resistant strain in the ATCC collection that is resistant to more than one class of preservatives due to the different mechanisms in how an isolate can be generated.

A13: Sure it is important to establish that the type strain you may use in specific dosage form is representative of the resistance of wild types found in product and the manufacturing environment but for practical reasons and the need to standardize the test you cannot routinely use multiple strains of one challenge organisms.

A14: I think that it is going to be difficult to select a type strain of Bcc for conducting a USP AET due to potential issues that there is no type strain that would not be representative of those Bcc isolates that are commonly obtained in manufacturing and antimicrobial resistance to various types of preservatives. For product development, I do question whether the USP AET would be the appropriate challenge test to conduct in the first place. For product development work, I'm in favor of using an in-house challenge test that is based upon the USP AET by using more strains (type and wild-type), sampling points, and more stringent challenge test criteria than what is currently in the method.

A15: This is excellent discussion and agree that early stages of development must include additional strains (in addition to the USP strains) and more stringent criteria or shorter sampling durations points to allow for some scale up and processing changes related decline in efficacy. However, I have fundamental issue with using mixed inoculum because that is a recipe for disaster and gives a very false sense of efficacy.

A16: I endorse [name redacted] point. Recall please the 51 microorganisms are all clinical isolates (but for 16404 - blueberry) of relatively ancient isolations (6538 dates back to around 1930's). Esp. in context nonsterile production of cosmetics and OTC drugs, expanded inocula (including plants isolates and more demanding time points better serve risk assessment.

A17: First you should identify yourself.

The <51> challenge organisms have a long history of successful use in developing non-sterile multiple-use products with robust preservative systems. The chapter encourages the use of additional challenge organisms based on your experience with relative products and the risk assessment.

A18: In reference to your statement concerning that USP Chapter 51 AET has been used successfully for many years in developing robust preservative systems in non-sterile products, I tend to disagree. By having a 2-log reduction in the counts at 14-days post inoculation, I do not consider this level of bacterial

reduction to be robust. I consider this 2-log reduction in bacteria at 14-days to be the bare minimum criteria for preservative adequacy of a non-sterile product formulation. From my experience in developing preservative systems and performing challenge testing on many different types of non-sterile products, I have the opinion that a robust preservative system for reducing bacteria is a 3-log reduction at 24 hours and a 4 to 5-log reduction at 7-days and thereafter post inoculation.

In the European Pharmacopeia 5.1.3 Efficacy of Antimicrobial Preservation Test, I believe that the A criteria for preservative adequacy is a 2-log reduction at 2-days and a 3-log reduction at 7-days for non-sterile products which is way more stringent that the current preservative adequacy criteria in USP Chapter 51 AET.

Because of the differences in the preservative log reductions between USP and EP, I suspect that this is one of the main reasons as to why no harmonization has occurred in this test method between the 2 pharmacopeias.

In reference to a previous comment by [name redacted], I think that he has a point concerning the origin of the 5 main challenge test organisms because they are clinical isolates. How applicable are using clinical isolates for conducting preservative challenge testing? I have previously raised questions as to whether each of the 5 identified challenge strains are applicable today for conducting challenge testing. If you have ever conducted microbial in-use studies of non-sterile products, you rarely find the presence of S. aureus isolates in a used product formulation unless the person that had used the product is a S. aureus carrier. Most Gram-positive cocci contamination isolates in used products is Staphylococcus epidermidis. In addition, you never find the presence of Escherichia coli in a used consumer product formulation.

From my perspective, I tend to believe that the whole USP Chapter 51 AET needs to be revised and updated. Besides changing the criteria for preservative adequacy from the current limit to make it more stringent, I would also change the types of organism that are being used to conduct challenge testing of non-sterile product formulations to the following:
Pseudomonas aeruginosa ATCC 9027
Burkholderia cepacia ATCC 25416
Staphylococcus epidermidis ATCC 12228
Pluralibacter gergoviae ATCC 33028
Klebsiella oxytoca or pneumonia ATCC type strains
Candida albicans ATCC 10231
Aspergillus brasiliensis ATCC 16404
Wild-type strains that have been obtained from microbial rejects of finished product

If this method were ever going to be used for product development work, I would also include a re-challenge step after sampling at 14-days.

Because most microbiologists are resistant to accept changes in their test methods, I believe that my above proposals are going to be opposed for the most part in changing the current USP Chapter 51 AET.

A19: I don’t know how I ended up with this long alpha numeric but will certainly sign my name in future posts.

51 provides consistent results to a level of preservative capacity that has limitations in some applications. Nevertheless, it is the go-to test – in the applications of protocol (EP,ISO, etc.) it’s the best-known and go-to test across industries. My focus has been cosmetic, household and industrial products - these folks use 51 for consistency, simplicity and as it's the test most readily offered by some commercial labs for the same reasons.

For cosmetics, I find it inadequate as titration of preservation to just meet topical criteria (2) is not reliable for consumer protection in-use – the nominal objective for cosmetic preservation. Appropriate (hopefully validated) addition of resistant (or capable of resistance) cepacia and the like offers confidence of consumer protection (recall Dan Brannan’s work). Still 51 is often used for cosmetics risk assessment. In the hands of some, this limitation is obscured as substantial preservative capacity typically formulated

makes 51 pro forma. Others apply 51 protocol with much more demanding performance criteria. It's also worth noting that risk assessment in context of large, high volume/high speed/nonsterile global manufacturing drives consideration of such isolates in technical and political sense. Business impact when encountered establishes painful memory. The resident microbe can be a reality , and the microbiologist has to address "never let this happen again" while resisting the addition of every troublesome isolate encountered anywhere in global facilities.

The other industries mentioned typically have limited internal micro knowledge/support and defer to commercial labs. As there isn't a test appropriate - they often end up with 51. For those products, the primary/only consideration is manufacturing contamination often with bugs we might think more or less extremophiles. Here 51 is pretty ineffective – unpreserved products typically pass quite well.

A20: Sure USP <51> is a minimum requirement for regulatory submissions and stability testing. Typically in product development in the pharmaceutical industry we combine its acceptance criteria with those of the Ph. Eur. test and based on knowledge of the product we add additional change organisms. We consider the totality of the formulation in terms of its preservative effectiveness.

Our objective is to prevent extrinsic microbial contamination that may overwhelm the preservative system and do harm to the recipients of our products.

A21: if you read my post I clearly said that we combined the acceptance criteria from the Ph. Eur. and USP tests when we were evaluating preservative systems.

However, the USP criteria is an absolute requirement for regulatory submission whereas the Ph. Eur. is not.

A22: Just because the USP criteria for preservative adequacy is a regulatory requirement, it doesn't mean that it is correct. For topical non-sterile product formulations, they are not normally used once every two weeks so that you must wait 14-days in order to have a 2-log reduction. Topical non-sterile product formulations are generally used at least once a day for a week or two in which there is the possibility of a greater chance of the product becoming contaminated during consumer usage. In this case, I think that it is more applicable to have a log-reduction for preservative adequacy that reflects the actual usage of the product.

A23: I am following your debate on USP Chapter 51 with interest. I always wondered: since you implemented the harmonization with EP of the release specifications of non-sterile products with numerical limits, but you have kept the abatement criteria in chapter 51 (harmonized test in execution since 2011, but not harmonized) if you have problems with out-of-bounds market recall more often than European products.

A24: What about the testing of Bcc for dry powders for oral suspensions. Please let me know. In my facility we have antibiotics as dry powders for oral suspensions.

A25: I have enjoyed the 'conversation' regarding Bcc and USP <51>. I have wondered how reflective USP <51> is of the "real' world, I understand it is meant to be 'standardized' test.
Since the microorganisms that would be contaminating consumer products etc would not have been growing on a petri dish, and thus challenging a preservative system using microbes grown on a petri dish does not represent the 'real' world, are there any suggestions/comments regarding how the challenge test could be made to reflect more 'real world' conditions or at least increase the robustness of the test. This would be of value during the product development stage.

In the past, we have taken advantage of the 'acid tolerance response' to evaluate the ability of challenge organisms to survive under desiccated storage conditions, with the goal being to create 'worst' case conditions. We did find that the challenge organisms grown under conditions that induced ATR did show significantly greater ability to survive under desiccated conditions when compared to those challenge organisms grown under 'standard' conditions.

A26: It's a good question and something that was partially addressed by Novartis for a rapid sterility application. The question is "how do we stress the bugs without killing them?" It's not easy and there are many ways to do it but I can't say I know of the best way to stress Bcc (or other bugs) in a non-sterile formulation environment. Our friend Michael Miller was also involved in this process so perhaps he can expand on trying to use real-world bugs. If you search "Novartis stressed organisms" you'll find the publications.

A27: Right that is why we extend the evaluations beyond the minimum requirements of the USP Test during product development.

I do like the in-use challenge testing used for cosmetics and OTC products.

A28: Cultivating inoculum in dilute (1:10) broth also establishes a more resistant challenge, esp. for Gram megative bacteria.
If cosmetics, consider an in-use challenge - e.g. https://aem.asm.org/content/53/8/1827.short.

A29: A comprehensive micro control approach would include challenge using compendia requirements, plus additional isolates maintained to keep the metabolic stress and appropriate in use test are key for preserving consumer products. This type of approach is not one size fits all but must be specific to product type and must take into account the packaging, storage and usage pattern.

A30: We have been using facility, process or product-recovered organisms for rapid method validations, in addition to compendial or ATCC type cultures. But in these cases, we are growing them up in the lab prior to use, and in some instances, stressing them to force a longer time to recover. The latter is done to establish a minimum incubation time for organisms that may take longer to replicate, under the conditions of the test.

So, I agree with [name redacted] that you can never truly replicate a “wild type” physiological state once you take it out of the wild environment and grow it in the lab. That being said, I used to inoculate fresh product with organisms obtained directly from a contaminated product container (to test the AET in non-contaminated formulations), without growing the organism on an agar plate first. This is the only way you can get as close to the wild type physiological condition as possible. But you would need contaminated material to accomplish this task, which most of us do not have.

If you wanted to stress organisms, you can use a sub-lethal heat treatment (50-60 deg C) for 1-10 minutes, until you have 50% loss in viability. This is for bacteria and vegetative organisms only. For spore formers, you can incubate in sterile water at 2-8 deg C for one week. This may prolong the germination phase. Again, these steps are to lengthen the time to replicate/germinate, but may not necessarily impact the organism’s susceptibility to a preservative or antimicrobial formulation.

Reporting result of MLT

In MLT we take 10 ml sample in 90 ml diluent. For total count by pour plate method, we pour 1 ml from above dilution in duplicate. If we do not found found any colony then we report theresult less than 10 cfu per ml because consider factor 10.

In case of membrane filtration method, if we filer 10ml sample from above dilution and after incubation we didn’t find any colony. And does not apply factor.
Than how we report results.
1. NIL or Absent per ml
2. Less than 1 per ml.

A1: Since 10mL of the diluted sample is equivalent to 1mL of sample, you would report the results as <1CFU/mL.

BCC testing

We manufacture non sterile topical Gel. We make this product once every two months.

We are performing USP <61> testing in house, but for USP <62>, we are sending it out to a lab. This absence of objectionable organism testing <62> now includes BCC complex.

My question is, do we need to include BCC for <61> ?

If yes, then perform growth promotion and system suitability test according to the <60> in the supplement?

A1: Technically USP <60> and <62> contain tests for specified microorganisms, They are too selective for screening for objectionable microorganisms. I would refer you to PDA Task Force responsible for the development of a Technical Report No. 67 *Exclusion of Objectionable Microorganisms from Non-sterile Pharmaceutical and OTC Drug Products, Medical Devices and Cosmetics* 2014 for guidance

For method suitability testing and growth promotion tests for the media if you follow the instructions in the USP chapter you have met the requirements of the test. There is no need to use Bcc.

A2: Not for USP 61. Just follow the required growth promotion and method suitability organisms prescribed in the chapter.

Area qualification requirement

I have question regarding area qualification in sterile manufacturing unit , Is there any guideline says we should do area qualification for 3 consecutive days or 3 different dates?
If anyone knows any supporting guidelines please share with me.

A1: I have found the following document to be extremely helpful regarding this topic. "The Environmental Monitoring Program in a GMP Environment" by Scott Sutton.

http://microbiologynetwork.com/doc/Sutton.jgxp_.14.3.pdf

Rapid microbiology test for MLT and Sterility

Please share your experience and guidance on Rapid microbiology testing for microbial enumeration test and sterility test by using instruments of different makes.

A1: I would recommend reviewing the extensive information provided at http://rapidmicromethods.com.

The website includes a product matrix with a summary of almost 100 rapid method technologies for quantitative, qualitative (sterility) and identification applications.

Also included is guidance on regulatory expectations, validation strategies, return on investment considerations and a very large reference page.

Lozenge

I would like to know should lozenge be classified to "Oromucosal use" or "Nonaqueous preparations for oral use" according to its route of administration in USP Chapter 1111? I always think that it should be classified to dosage form for Oromucosal use but someone argue that it should be classified to product of Nonaqueous preparations for oral use. Any reference for such classification? Thanks.

A1: Technically, oral ingestible but since it stays in mucosal membrane contact for longer period and Oromucosal requirements are more stringent, I would apply the Oromucosal criteria.

Performance Qualification/Non-Viable and Viable

Please educate me as to how one should conduct performance qualification on LSAPC (light scattering airborne particle counters) and sieve impaction microbial air samplers, especially considering inherent sampling limitations involved in both instruments (e.g. counting efficiency for LSAPC and d50 for MAS).

A1: You don't need to perform a PQ for the MAS-100 NT because we've done it for you in controlled conditions. There is no regulatory expectation that you would need to perform a PQ in a true sense (biological and physical efficiencies) because it's not practical and air is not homogenous so it's almost impossible to get consistent data to show the true recoveries of the instrument. We do have an IQ/OQ package and a validation study showing recoveries under controlled conditions that is accepted by regulatory bodies. Our samplers are validated to ISO 14698 standards (Now EN 17141).

A2: I assume the LSAPC instruments you are referring to are intrinsic fluorescence systems such as the BioVigilant IMD-A, BioLaz and BioTrak.

You would run your LSAPC and MAS side by side and collect the same volume of air and do a comparison of the results. But you are correct, there are limitations:

The LSAPC are more sensitive and will detect more microorganisms, if they are present. The dirtier the area tested (e.g., Grade D or uncontrolled), the greater the difference in recovery you may find. This may be due to the detection of stressed or dormant organisms in the LSAPC.
The MAS (and most other conventional air samplers) have terrible d50 values and collection efficiencies. This may account for lower counts compared with the LSAPC.
The time to collect the same volume of air may be different. Some LSAPC systems require 35 minutes to collect a 1 liter sample, where the MAS can do the same in less time. This may also present a limitation in that one system will continue to operate when the other has stopped. For this reason, you may be able to justify a correction factor to match the sampling times.

I published some of the first studies using these instruments in 2009. They are both available in PDF on the reference page at rapidmicromethods.com. Here is the link (just look for the papers and download): http://rapidmicromethods.com/files/references.php.

The first paper compares the MAS-100 with the BioVigilant IMD-A in cleanrooms. I included an extensive discussion on collection efficiency and d50 values: 2009. Miller, M.J.; Lindsay, H.; Valverde-Ventura, R.; O'Connor, M.J. Evaluation of the BioVigilant IMD-A, a novel optical spectroscopy technology for the continuous and real-time environmental monitoring of viable and nonviable particles. Part I: Review of the technology and comparative studies with conventional methods. PDA Journal of Pharmaceutical Science and Technology 63(3): 244-257.

The second paper describes the performance of the system in an isolator environment: 2009. Miller, M.J.; Walsh, M.R.; Shrake, J.L.; Dukes, R.E.; Hill, D.B. Evaluation of the BioVigilant IMD-A, a novel optical spectroscopy technology for the continuous and real-time environmental monitoring of viable and nonviable particles. Part II: Case studies in environmental monitoring during aseptic filling, intervention assessments and glove integrity testing in manufacturing isolators. PDA Journal of Pharmaceutical Science and Technology 63(3): 258-282.

A3: I am going to respectfully disagree with some comments you received.

You do not have to complete the VALIDATION of your air sampler, which the manufacturer (or 3rd party) has performed. The manufacturer will provide you with this validation statement of physical and biological efficiency performance of their equipment. If you refer to BS EN 17141 (which will soon become as ISO standard that replaces ISO 14698) as well as PHSS Monograph 20, they recommend a d50 value of less

than 2.0 um (the size particle of which the sampler has been shown to capture at least 50% of). The MAS series of equipment satisfy that recommendation.

From a PQ perspective you would simply begin to sample, using the MAS, in your specific area(s) to collect enough data to establish your viable active air alert level. Additionally you would conduct media desiccation studies that demonstrates your selected agar (e.g. TSA, SDA) can support growth throughout the sampling, hold time and incubation period (i.e. you do not pull "potato chip " plates out of the incubator). This is effectively a simple post-sampling and post-incubation growth promotion. Some manufactures will provide you with this data as well, however, you should conduct this under your unique environmental conditions.

A PQ on a particle counter would be the same, where you are statistically deriving the alert level based on your trended data.

A4: I believe it would be wise to ask Jason to better clarify his original question. I took it as how to perform a PQ on an intrinsic fluorescence air sampler and how to compare this to the stanrda method (in his case, the MAS-100). This is how I interpreted his question when formulating my reply.

[name redacted], can you please clarify for us if this was the intent of your original question to the group?

A5: I am full aligned with the comments perfectly written hereunder by [name redacted], with all my respect.

A6: I take this to be part of the EMPQ, not necessarily just the instrument. As written, a PQ of the instrument alone isn't required, but will be used in the EMPQ to start tracking and trending levels. I suppose this is the junction of the instrument operating as it should (OQ) and operating within the framework of the EMPQ plan that only uses the instrument as part of the entire plan. Microbiology makes everything gray.

Transfer of method from another laboratory

i have a customer that wants us to test their raw materials and primary packaging component items. My company has already performed method suitability previously (for another customer) on either the same raw material or similar shape/size components with same/similar materials of construction

my questions are,

1. For the raw materials does a method suitability need to be performed again? As even though the analytical tests associated would comply with material specifications does the micro MLT suitability need to be repeated or can you justify that if it works for one supplier and analytically it is the same then it will work for any supplier of the same material.
2. Instead of repeating a full suitability with 3 batches could a verification of sorts be performed where only 1 batch is confirmed to have acceptable recoveries of organisms expected?

For the component method.
Again can the method be interchangeable for any similar type of component so no need to do suitability and just have a written justification?
As a minimum could i again use the approach that a verification with 1 batch will be done?

or must you perform full method suitability for each test item?

Just as an additional question, is there ever a way that you dont need to do method suitability if you were transferring someones validated method and you just used that to test the materials as they will be supplied by the same supplier that they used?
Why must we repeat it all again?

A1: If you were testing for your own internal manufacturing use - reduced testing might be justifiable based on extensive evaluation of historic trends.
But if I have understood correctly and you are performing 3rd party contract testing for different clients - in my opinion you should complete the defined testing requirement in full.
The issues in order of significance are;
1) Legal / Contractual / Intellectual Property Issues.
2) Workload issues in establishing that materials / articles are suitably comparable i.e. unless produced by the same manufacturing site & process.

Sure, in theory if the materials & components and methods were "identical" one could attempt to justify reduced testing, it makes theoretical sense, but the rub is "who" owns and has control of the data.

The client should expect that you fully perform the relevant testing specific to them and have complete access to a discrete contemporaneous and integral data set unless they specify otherwise.

If I outsource a test, I want to have complete control of the data and the data should be current. Use of "historic data" to support test decisions generally requires that such data is legally free to use and should be suitably recognised as sound (i.e. peer reviewed etc).
Quality Agreements should require that the requested test input and outputs belong to the contract giver and cannot be shared or used by any other party without permission.

Were a second company to use another's data (with or without permission) the scope of any subsequent investigation of an adverse regulatory / legal exposure that included reference to the source data, could potentially extend to the original data owner and associated operations.
A legal quagmire for all.

Regarding conducting the requirement for System Suitability in a new lab space even where all reagents, materials and methods were identical, I would still anticipate evidence of suitable training of receiving analysts (new lab) by current users (transferring lab) using the defined method during the transfer process. Suitably supervised, documented, and successful training runs establish competence and effectively equate to System Suitability. You are determining the test suitability in your hands, under your test conditions, i.e., facility, personnel, and equipment as well as reagents & materials. So, you either call it Training or Suitability Testing or Suitability Training, but it still should be done, in my opinion.

A2: If the RMs are identical then there is no need to perform another suitability study. The catch is that the different suppliers must provide identical material and if you cannot confirm this, you should perform a risk assessment to determine if suitability should be repeated. But in the event you have to perform another suitability study, if your risk assessment shows there is not a difference in RMs that would impact the micro test, then you may be able to justify performing suitability on a single batch. I would just be careful when working with a new supplier you have no prior relationship with, and at the very least, perform your analytical/identity tests on at least three batches.

You can use the same guidance above for components.

If all things remain equal (same RM from the same supplier), then you should not need to repeat the suitability. However, you need to ensure the receiving lab is able to perform the routine testing adequately (i.e., using the validated method incorrectly is not good). Therefore, at a minimum, I would suggest auditing the lab for their prior suitability studies and match these with the routine testing they currently conduct. This way you are confirming your method can be translated into a routine test without issue.

I am certain some of my seasoned colleagues will chime in, but the guidance I provided are examples of what you may consider, depending on your situation and the outcome of your risk assessments.

Culture suspension

As per USP/BP/IP prepare the culture suspension in buffered sodium chloride peptone pH 7.0. And use with in 4 hrs. If we are validating for storage period, it is possible bacteria remain same because they can multiply as peptone is present.

In pharma industry, people use 0.9 percent normal saline for prepare the culture suspension as saline maintain cell viability. Please suggest that what should use for culture suspension either 0.9 percent saline or buffered sodium chloride peptone pH 7.0 for approx. 7 days storage at 2 to 8 degree.

USP 62 Suitability test

I perform the suitability test for E.coli as described below:

10 ml sample 1:10 (corresponding to 1 g of product)
+1 ml 10^4 CFU E.coli
+89 ml CSDB
Mix and incubate
Is it correct?

Non Viable and Viable Air samples

I am currently working on EM program and am trying to establish Volumes of Air taken for both Viable and Non-Viable particulates. I know "797" mentions 400-1000 Liters should be tested for Viable air and a cubic meter (1000L) for Non- Viable air. However I am looking at a way to cut that time down because of the amount of areas in one room that need to be monitored. I haven't found it in any guidance's anywhere but does anybody know if or how I can do this and still maintain standards.

A1: Many active air sampling devices can collect 1 cubic meter (1000 L) in 10 minutes. I take it you use a tripod for the air sampler. You could review the EM history and limit the sampler to worse case locations.

A2: Our procedures called out to sample for 1000 volume. As you know to collect 1000 volume will take approximately 30 min per sample, we reduced the volume to 500 and we are multiplying the result by factor of 2.
We performed an study to show the equivalency between 1000 volume result and 500 volume result x2. We did collect data from various area (about 20 points) on both ways and did comparison. The variation was <0.05%.

A3: Our procedures called out to sample for 1000 volume. As you know to > collect 1000 volume will take approximately 30 min per sample, we reduced > the volume to 500 and we are multiplying the result by factor of 2. > We performed an study to show the equivalency between 1000 volume result > and 500 volume result x2. We did collect data from various area (about 20 > points) on both ways and did comparison. The variation was <0.05%.

A4: For monitoring the same sample volume as used for ISO certification need not be used. A risk based monitoring plan of an area (clean room or clean air device) would yield a more manageable sampling programme and be more in line with the risks posed. I would suggest that the PDA Technical Report 13 be part of your review.

FW: sample size vs sample dilution

I would appreciate any thoughts anyone has to offer for following topic.

“USP 61 tells to do your sample preparation using 10g of sample. Is it important to use 10g or can we use more or less that 10g as long as we are doing 1:10 dilution.”

How important is the sample size?

How important is the sample dilution?

If one has to choose which one is should be preferred and why?

A1: The sample size will determine the sensitivity and the responsiveness of the test.

The dilution may overcome the sample effect and bring the test into a countable range.

A2: In USP 61 it says to use 10g product is there a reason for that? Not 11g not 25 g not 1g. What is the importance and is it okay to not follow it?

USP 61 and 62 are linked. Like you use 10g sample into 90ml broth do your plating then transfer 10ml into 90ml TSB for USP 62 (making further 1:100 dilution =1g of sample when go in incubator for qualitative test) right?

A3: The USP recommends a minimum sample size 10g. With 10 g a 1:10 dilution will give you a 1 g test sample.

USP <61> and <62> method suitability

When performing suitability testing and you need to obtain your collections of 10g/ml of product to perform the 1:10 dilution and then your enumeration or enrichment suitability tests, do you need to use filled devices or can you use bulk formulation?

I have always done it by obtaining the sufficient amount of filled devices and pooling them together to make sufficient amount to execute the testing for a batch. This could end up being hundreds of devices. Having a re-think i cant see any detail within the pharmacopoeias or think of a reason why we need to use filled devices to prove recovery from the product. we are not concerned about the manufacturing process/ environment adding contamination at this stage.

Any guidance references or peoples opinions are greatly appreciated.

A1: The USP provides a general test method employed to demonstrate a compendial article meets the monograph requirement.

The suitability tests can be conducted on the bulk solution or pooled finished product.

The pharmacopeia and the regulatory cannot provide guidance for every circumstance, so you have to rely on your best judgment.

A2: One could always argue that the final container materials may contain some amount of leachable substances that need to be accounted for in the suitability test. I don't really subscribe to that argument myself, but there's always someone who will make that argument. As I recall your situation, the glass vials and rubber stoppers used for your product would be what that person points to.

On the matter of sample size for suitability, my personal opinion is that method suitability is not locked to the 10mL/g/patches like the routine testing is. As long as the ratio is kept constant I would accept that data. 1 mL of a 1:10 dilution contains the same amount of material whether it was pulled from 10mL or 100mL preparation. Different opinions are welcome.

On a related topic, USP <610> has some information you might be looking for with respect to testing bulk intermediate rather than the filled units for single-dose nasal sprays.

A3: I didn't quite understand the question perhaps.
1 if it is for the routine test yes, 10 g or ml must be used, even if in EP it is written that in the case of small batches different and smaller quantities are allowed
2 if it is for the suitability test of the method, let's not forget that the microorganisms can also be added directly into the petri dish, as long as the ratio of 1% is respected, so if you inoculate 1 ml of prepared sample in the plate, you must inoculate a maximum of 10 microliters of bacterial suspension containing about 100 CFU.
3 the tests must certainly start from the product in the primary container if we talk about release tests, for suitability it may not be necessary, but then it could also be said that the suitability was not performed exactly like the routine test.

A4: I realize I'm resurrecting an older conversation, but I wanted to ask about one of the comments below.

The first sentence of the inoculation and dilution section of USP 61 that states: "Add to the sample prepared as directed above and to a control (with no test material included) a sufficient volume of the microbial suspension to obtain an inoculum of not more than 100 cfu. The volume of the suspension of the inoculum should not exceed 1% of the volume of diluted product." Since this language comes before the "RECOVERY OF MICROORGANISMS IN THE PRESENCE OF PRODUCT" section where media is added to the plate in the plate count method, we've interpreted that as sample preparation, inoculate, and then plate.

Is there any such allowance in the USP such that "microorganisms can also be added directly into the petri dish, as long as the ratio of 1% is respected" during the inoculation for suitability?

A5: You can find the answer to your question in the USP <61> FAQ published by USP, which is available if you go to the USP.org website and select the FAQs menu. Specifically, the answer you seek is #11 in the USP <61> FAQ.

A6: I'm trying to wrap my head around the impact of this method. If there was insufficient organism recovery after plating an inoculated sample preparation, yet plating the preparation and the inoculum could give sufficient recovery, wouldn't that give a false sense of efficacy? If they were equivalent, I would expect similar results.

A7: Yes - If there were a discrepancy between the results from a liquid sample inoculation or that observed when inoculation occurs later during product plating (e.g. pour plate technique), it would indicate that the product inactivation / neutralisation step was not effective.
I have always seen the test as having two distinct phases, Inactivation / Neutralisation which is if part of sample preparation and is a pre-requisite of inoculation.
Establishing an effective Neutralisation / Inactivation protocol would then take place prior to the actual suitability test and as I believe you are highlighting, would be best done using a diluent/broth/ product mix system (worst case exposure) , with subsequent progression to the relevant product suitability testing technique being used locally, (e.g. pour plating in your test scenario).

Microbial reference culture passage

We procure the lyophilized culture of passage 1 from authentic culture bank. If we grown in scdm for purity and identification then we consider it passage 2 or remain passage 1. After confirmation transfer from broth to fresh agar slant than it consider passage 2 or passage 3. This passage phenomenon is right. Please suggest.

A1: When the inoculum is transferred from lyo form to fresh media will become passage 2. Again from scdm to other fresh media or slangs will become passage 3.

A2: Passage 3 is right.

A3: Anytime you grow the organism, it is a considered the next passage.

For example, if you took a loopful of the growth from the agar slant (passage 3) and put into nutrient broth that was then incubated, the growth from the broth would be passage 4. However, if you took a loopful from the agar slant (passage 3) and put into a diluent such as saline to perform dilutions, that would not be an additional passage (passage 3).

USP <51> PET test for Injection

I have been reading the conversations regarding USP <51> and EP requirements/criteria. Here I have different issue and hopping to get some guidance on it.

Our customer is developing an oil based formulation for a multidose parenteral product, that contains no water but preservative. As the injection is a multidose product containing preservative, we need to determine the effective content of the added preservative using USP <51> PET test.

We are receiving PET test results that do not comply with USP <51> specifically for one challenge organism E. coli, for day 14 time point. The criteria is NLT log 3 reduction from initial day 0 count for bacteria. We are getting log 2.6 and 2.3 reduction respectively for 90 % and 100 % preservative content. We obtained 3.3 log reduction at 80 % preservative content. We are not sure, if this is could be just testing lab error or DP formulation.

Any suggestions to obtained NLT 3 log reduction for all bacterial challenge?

A1: Interesting problem.

Firstly the Ph. Eur. AET limits the use of E.coli as a challenge organism to oral liquids but the UsP test uses E. coli for all aqueous, multiple-use dosage forms.Is the aqueous, i.e., has a water activity >0.6?
So the higher the preservative level the less effective it is? Does the addition of the preservative change the attributes of the formulation in any way?

A2: Challenging anhydrous, oil-based materiual is problematic and data of limited to no relevance to micro risk. As Steve Schnittger and Jack Sabourin described*, suspended droplets of inoculum may not physical contact with preservative - here, esp. if an unfavorable octanol/water coeff. Different results may be attributable to inconsistent mixing emulsification of inoculum between challenges - just the variability of test.

A3: Is it just one time testing data point or average of multiple testing?

I recall similar experience where in 80% preservative level had higher activity as compared to 90 or 100% consistently over multiple testing. The batches were made by diluting all ingredients and not only the preservative- thereby resulting in the final pH of the product being more acidic for 80% preservative level. The product was preserved with organic acid preservative, which were more active at lower ph. Worthwhile to investigate- if your results are consistent and if all batches are identical except for the level of preservative.

A4: Please confirm as to whether your preservative has solubility in the oil of your formulation. I‘ m also assuming that you are preparing your preservative inoculums by using a water based diluent. It is very possible that the preservative is not moving from the oil portion of the formulation into the water droplets of the inoculum due to partition coefficient of the preservative in which you are having lower log reductions. To show proper log reductions, you may need to prepare your inoculums by using the same oil as the formulation.

A5: No water content in the formulation, and no moisture detection test performed. So cannot really say if there any water activity.

The oil changes slight color at the addition of preservative, no other physical changes in the solution. Solution remains clear.

A6: I would agree with [name redacted] that the inocula add water to the formulation. The preservative will partition between the oil-water phases which will affect the antimicrobial activity.

reserve samples

Reserve samples are kept in a sufficient quantity to perform two retests for chemical tests.
Do I also have to keep a quantity of samples to do two retests of microbiological tests?
This relates to raw materials and final products.

A1: The reserve sample should be enough to repeat all tests in triplicate (chemical and microbiological) required per specification and should be an integral part of the material and product specification to ensure the requirement is followed consistently.

A2: I will suggest keeping enough retain samples to test your product fully three times unopened. The secondary purpose of reserve samples is to allow a regulatory authority, you, and a third party lab to perform all testing on your retain samples. This testing is in addition to the testing that you perform according to the CFR and your internal procedures. I typically see that many firms fail to hold sufficient quantities of unopened product in retain, until they experience a severe adverse event. Sending out opened and exposed product may expose your product to microbial contamination and oxidation.

I am assisting several firms/consumer in investigating an adverse event situation. The occasion happened 18 months, because the courts take so much time. I'm asking for retain samples to test as a third party and the firm has no unopened containers to defend themselves. It's never a position that I ever suggest to any firm.

By the way, many of my current projects are the least regulated - cosmetics and dietary supplements. It is so scary, because I lack the ability to defend by testing and verifying the contents. In addition, these firms failed to perform stability testing. These firms are learning their requirements, even when the regulation does not require the regimen of testing. These lawsuits are several million dollars each.

Funny, after such events, these firms start holding 7+ sets of retain samples. Three sets to test internally and four sets to hold for exposure. I also suggest holding these products for slightly longer than CFR requirements, or answer why you you don't need to test. In some cases, it is better to destroy your retains, because you fully understand your shelf life.

A3: 21 CFR 211.170 Reverse Samples states "1 a) An appropriately identified reserve sample that is representative of each lot in each shipment of active ingredient shall be retained. The reserve sample consists of at least twice the quantity necessary for all the tests required to determine whether the active ingredient meets its established specifications, except for sterility and pyrogens testing" 3. b) for drug products repeats this statement.

A4: I agree with [name redacted] that holding several additional samples is a must.

The fact that you are being Proactive here and not reactive to an after the fact event is encouraging.

The number of samples you hold for each batch of product manufactured should allow you to perform adequate analysis to confirm or discredit original results.

In many cases your need to retest will not coincide with the chemical labs need to perform retests, there process will also differ in the number of retests required.

Firstly, for Microbiological analysis it is good practice to always obtain twice the amount or double the number of containers that you need to perform initial tests.

This is in addition to the additional samples that the chemical lab may, as standard, obtain.

Secondly, at manufacture you should always have a documented process of retaining samples of all batches which will cover the list of possible issues.

Issues such as test result anomalies, both micro and chemical, quality assurance investigations or customer complaints.

Thirdly, as any batch should not be released to market prior to the completion of official documentation (batch release), with parametric release based on sound risk assessment being the exception to the rule, it is reasonable to assume that you have access to the batch under storage wherever and however that may be.

Although this can be a task in itself if you do not have the correct procedures in place it does provide a fail safe to obtaining additional units of suspect batches.

In answer to your question, you should hold adequate amount of samples that you feel enables you to complete investigations regarding your batch with anomalous results. But as a minimum that should be documented in your retained sample procedure, your lab procedure and your work order (so those on the filling lines know how many to take).

Use of hand sanitiser as part of gowning procedure to classified cleanrooms

As part of the gowning procedure to enter classified cleanrooms following hand washing with antimicrobial soap we apply hand sanitiser (alcohol gel), then don the cleanroom suits etc..

What testing must we do on the hand sanitiser and the alcohol gel that are used to wash and sanitise hands before entering a GMP cleanroom area?
are we ok to accept manufacturers tests against ISO standards or must we perform some type of in-situ evaluation of our own on these materials?
The ones we are using have been in place for many years and i don't think any testing has ever been done and I'm assessing if we have a gap.

If we are ok to use the manufacturers CofA and don't require to do any testing, an additional question is; if we decide to change any of these products to a new supplier do we need to do any type of assessment to show that there has been no impact to the environmental quality within the GMP areas by monitoring some EM plate trends or perform some finger plates etc? just to give us some confidence of no impact.

What are your thoughts on non-alcohol sanitisation gel?

A1: Reports suggest that hand washing is as effective or even better as using a hand sanitizer so it seems superfluous to do both. Some companies view hand washing stations as reservoirs of Gram-negative bacteria so vote for hand sanitizers. Both methods only reduce skin counts by a log or 2 and remove transient bacteria.Hand washing may be superior in removing dirt and fecal material.

A2: It has been my experience that it is not required to qualify the hand sanitizer as you would a disinfectant. We have been asked by regulators and have been successful in defending our position.

Part of the defense is that in critical areas, the hands are covered with gloves, which are subsequently sanitized with sterile 70% IPA. There may be some literature out there to reference.

A3: The EU requires testing on the cleaning solutions used in your cleanrooms (including IPA). I would imagine they would look for the same thing here. Remember that alcohol does not inhibit Bacillus so you could have a contaminated container that would transfer to the employee. [Unfortunately, I experienced this at one of my earliest jobs... the IPA on my EM cart ended up contaminating the sterile area. The

contamination came from improper sterilization of the carboy we refilled our bottles from. The company had to shutdown the area to clean with sporicidal agents... an experience you never forget!].

A4: I'll add a real-world story to yours as well. In college we were performing molecular biology experiments and everybody failed...even the control! The one thing in common was the spreader glass sticks that sit in alcohol. The alcohol was NEVER changed and mold/Bacillus contaminated everybody's experiment. It's a lesson that bears repeating.

A5: Your story brings back memories of the old "hockey stick" method. I do remember that when we removed the glass "hockey sticks" from the alcohol, we would place it into a bunsen burner flame, let the flame burn out, before proceeding to use it for plating. Interesting that your experience (assuming you flamed the glass) did not kill the Bacillus spores/mold. MICROBES RULE.

A6: Yes I investigated a BI failure and found that the device used to crack open the ampule was being decontaminated in a reusable alcohol solution. As I understood the implications, it took me a couple of minutes to pin-point the cause.

Water conductivity lab samples

We have recently purchased a new conductivity meter for our microbiology lab. We have found that with the new meter the samples take over 5 minutes to stabilize and get a reading. I contacted the vendor and was told that considering the purity of the samples this is not unusual. It seems excessive to me. Can anyone tell me what a normal time to stabilization is for purified water and WFI?

A1: It is (almost certainly) not the conductivity instrument that is slow, rather it is the equilibration of the sample with air. For virtually any liquid other than high purity water, the signal stability should be quick, seconds or a minute or 2, assuming a stable temperature.

For high purity pharmaceutical waters, the sample is not saturated with air while it is in the water system. When collecting the sample, the water starts absorbing air, specifically CO2. When the sample is transferred to the container with the conductivity sensor, the sample keeps absorbing CO2 until the concentration of the dissolved CO2 in the water equilibrates with the concentration of the CO2 in the air, a.k.a. Henry's Law.

Why is the CO2 important? The CO2 reacts with water, forming carbonic acid, which immediately dissociates to the hydrogen and bicarbonate ions. These ions are conductive, and this is why the conductivity is not stable. It is likely to be rising too. But the instability is due to the ingress of air into the water. Stirring the solution, to make some agitation, would speed thing up a little - this will accelerate the mixing of air with the water. Also, you will see the conductivity rise from 0.1, or 0.2 uS/cm (or whatever the starting conductivity is) to anywhere from 0.6-1.2 uS/cm due to the amount of CO2 ingress. It just depends on the amount of CO2 in your environment - and you cannot control this.

Also, be aware not to breathe on the water sample. The CO2 from your exhaling will cause the CO2, and hence the conductivity, to rise.

A2: You could use glass bottles with ground glass stoppers for sample collection.

Although it is useful to have a lab capability for conductivity and TOC measurement, routine measurement should be conducted inline.With waterborne biofluorescent particle monitoring I see the industry eliminating routine microbial counts as well.

A3: Perhaps you will have several answers to this issue and you could get a good understanding, I would be interested to hear the overall consensus.

For my part I have asked individuals who perform this test in our quality department and was informed that 5 minutes is not unreasonable although you wouldn't expect it regularly.

Replies were from 5 seconds to, it never stabilised.

When asked if a 5 minute stabilisation would 'raise eyebrows' the reply was a resounding No.

Water sampling plan for purified water

We have a hot (80-85oC) purified water system ring and sample all of the locations against an approved plan.
We have a non-sterile aqueous product and the water feed to the manufacturing vessel is sampled in line with the approved plan, once per 2 weeks. In addition to the sampling plan we also take a sample of the water used to manufacture every batch and this result is included in the batch file along with the MLT results for the filled product.

I am thinking that the additional batch sampling is excessive and not necessary and all that we need is the routine monitoring plan and results to be confident in the routine delivery of acceptable water quality. All historical trends from the routine and batch samples are the same and zero cfu/ml
What are peoples thoughts on this process and the need or not to perform batch associated additional samples for this type of product?
Is there a regulatory expectation to take batch samples?

A1: The additional batch sampling is not necessary and there is no such requirement in the pharmaceutical guidelines. You can prepare a risk assessment (based on FMEA) showing that the additional batch sampling is unnecessary and the risk of a negative impact is low. You may include also your historical data. The other option is to reduce the testing frequency from each batch to every 10th batch.

A2: No need to test per batch. But I would increase your routine sampling plan to more then once every two weeks. And consider testing for BCC. This seems to be. Sticking point with FDA especially for water used to make no sterile aqueous products.

A3: Depends on product type and risk evaluation if mitigation steps failed. Typically, you have limits based on a compendial where your product is sold. Once validated, those limits you need a sampling plan to mitigate risk and product test to confirm risk is mitigated. Once you have history that your system is in control you can go back to your risk evaluation and see if you can cut back on monitoring. Product will still need to be tested for release.Once done, you then need to be aware any limit (i.e count our type of microorganisms) exceeded in your monitoring plan, you may need to revaluate risk plan to be sure product testing is in compliant to mitigate risk. Then, you will need to have corrective action such as containment, increase sampling, and monitor the results for a period of time to understand if it systemic or isolated excursion. This is a continuous improvement procees as the exursions will occur. Hopefully, not a lot.

A4: In reference to your post, I would not recommend sampling of use-points every two weeks for an ambient circulating purified water system. However, I do understand your reasoning as to why the frequency of sampling is every two weeks because the hot circulating water would be bactericidal towards mesophilic water organisms. I'm assuming the frequency of sampling has been established from Phase II of the validation protocol of the water system and is supported by microbial testing data. In general, I have found that there was the absence of organisms in samples that had been taken from a hot water circulating purified water system. From a practical point of view, I would not worry about the presence of Burkholderia cepacia complex in a hot purified water circulating system. It is highly unlikely that biofilms would ever form in these types of systems. If you have validated the hot water purified water system, there is no need to take samples of the water for each batch that is made.

A5: Although I agree with your synopsis in that you are going above and beyond the regulatory requirements you have a small problem.

The batch test of the water going into the product is a documented procedure that you have created.

You will have to provide a justification to stop doing that.

Fortunately if you have adequate data to support that change it should be a relatively easy fix.

Q. Why was the procedure introduced in the first place?
Q. Did your water supply have a history of delivering questionable levels of contamination with it?
Q. Do you see non-water associated organisms in your results?
Q. Have you ever isolated water associated organisms (or those isolated from your water analysis) in the product?

There is no regulatory demand for you to perform this additional analysis unless you have reasonable doubt regarding the quality of your water supply or the batch manufacture process.

Risk assess the quality of the system, the quality of the water coming out of it and the quality of the batches being created with it.

That should be enough to support your reason for change before you present it to a senior quality member/QP.

However, you may also want to check any product licensing to see if the process has been mentioned on them, if so you will need to apply to your regulatory authority to change any licenses that include the process, which could become expensive.

If there is no mention of the process on the licenses then your risk assessed reason for change will suffice (provided it shows that by not performing the additional test you do not increase the risk to the product).

A6: Thanks for your comment. The finished product is tested for BCC, are you also suggesting to test the water also? I know that there has been a lot of discussion in this forum that <60> should only be for the product but it does mention testing water and seems to muddy the water.

Would i need to do a method suitability following <60> using the PW to show recovery of BCC or an internal study by spiking PW samples with <100cfu, hold for a few hours and filtering (R2A) to compare recovery rates against control count plates? but then we are not using the compendial method and media.

A7: No need to test batch samples. But include the sampling of water from manufacturing sampling points into your sampling plan at least twice per week. Alternatively sampling& testing need to be done from all other points including raw water.

A8: There is a requirement.

Per USP 1231 6.1.2:

6.1.2 QC SAMPLING QC sampling is intended to reflect the quality of water that is being used. These samples should be collected at the true point of use; that is, where the water is delivered for use, not where it leaves the water system. QC sampling must utilize that same delivery path and components utilized for a water transfer during actual water use. This includes the same valves, hoses, heat exchangers, flow totalizers, hard-piped connections, and other components utilized during water use.

In addition to the water transfer components, QC sampling must also use the same water transfer process employed during water use, including the same pre-use outlet and delivery path flushing procedure and the same outlet, fitting, and hose sanitization practices employed during actual water use. The water delivery process and components used for QC sampling must be identical to manufacturing practices at every system outlet for the QC sample to mimic the quality of water being used by accumulating the same chemical and microbial contaminant levels it would during actual use from that outlet location.

Where permanent connections from the water system to equipment are present, accommodation should be made in the design to collect samples from locations as close to the equipment as possible. For example, samples can be collected from special sample ports or other valves near the equipment connection that allow the collected water sample to accurately reflect the water quality that is used. Where the water transfer conduit is designed and/or definitively treated to eliminate all contaminating influences prior to water transfer through that conduit, PC sampling locations within the distribution system can reflect the quality of the water that is actually used for QC purposes at those permanent connections. However, the success of the design and treatments intended to eliminate these contaminating influences must be verified. This is typically done during water system validation.

Where routine water use practices involve contamination-prone activities, such as no pre-use flushing or poor hose storage/sanitization/replacement practices, these water use practices should be improved to reduce the potential for delivering contaminated water from the water system and for unacceptable QC sample testing results that reflect that same contamination.

A9: Note: USP <1231> is general informational chapter that provides guidance and is not a GMP requirement.

A10: There is no requirement to routinely test for BCC in water. The consideration is whether you have recovered BCC from process or finished product. Then I would definitely do some sampling to see if the source is associated with the water system (generation skid components, sampling ports, use points, loop, tanks, return, etc.).

As for the test method, you can use procedure as described in USP 60 without issue. And although you may not think that water needs to show suitability, it is not that difficult to do and you will have the data on hand in case you are asked. I would not spend time doing suitability for a quantitative recovery of BCC but rather, would focus on the detection of low levels of BCC qualitatively, using the enrichment broth and subculture onto BCSA. That should be sufficient. Michael

Reg <1111> Actual Limits

Kindly suggest in USP Chapter Limits for oral solid dosage forms are as follows;

1) 10 -3= 2000 cfu
2) 10 -2= 200 cfu
3) 10 -1= 20 cfu

But in routine practices in all product specifications it is as follows;

1) 10 -3= 1000 cfu
2) 10 -2= 100 cfu
3) 10 -1= 10 cfu

Then what is the meaning that given in USP?

A1: Actually the TAMC specification are not more than 10^3 CFU/g with the maximum acceptable limit not more than 2000 CFU/g.

A2: As we know that the recovery limit for microbes is 50% to 200%. So 10^3 is interpreted as 2000 in pharmacopoeias ; Most companies set their specification limit as 1000 because it ensures that even at 50% detection/recovery of microbes it won't cross the limit set by pharmacopoeias.

Area qualification requirement - CRAQ Programs

Re-focusing on a recent post thread:
What would relevant contributors consider to be their top 5 references - Guidelines, Recommendations, Codes of Practice etc, for conducting initial "baseline" Clean Room Area Qualification (CRAQ) program with an emphasis on Microbiological elements.
In response to a previous query, Scott Sutton's article in the J GXP Compliance 2010 was cited and provides some useful indications and starting points.
While most references I have come across focus on Air Quality test requirements/sample point selection,/ acceptance criteria / action alert determinations and Routine EM program Guidance etc., all of which are adequately dealt with in ISO standards such as 14644, relevant Pharmacopeia, PDA guides etc., there appears to be comparatively few information sources covering the nature & scope of the Cleanroom Area Qualification (CRAQ) Program itself, in particular;
Run Number & Frequency (consecutive shifts / days etc/ weeks , seasonal etc), Duration, and the accepted level of associated Microbial Identification Work (% isolate screening v full ID) per different Area Classification etc.
Over the years I have inherited various ad-hoc programs, (some of dubious pedigree) that vary greatly in the level of resource input (often with little scientific justification).
Maybe I have missed suitable sources that were in plain sight or perhaps there is little definitive guidance, and one must extract relevant bits from the FDA Aseptic Guidance, USP <1116>, Eudralex Vol 4 Annex 1, PDA etc., as I have done in the past.
Just wondering what documents others are using in developing or justifying initial CRAQ programs.

A1: Those are some pretty in-depth questions! I think this is synonymous with EMPQ because you're focusing on the microbiology aspect of cleanroom qualification. Fred Ayers did a good job explaining his rationale for an EMPQ at the PDA Micro show a couple years ago. If you search "EMPQ", an article in APR comes up that discusses this topic. Crystal Booth also published an article on Pharmaceuticalonline.com that is a great resource. Finally, BioPhorum has create a risk assessment tool that is very useful for this purpose as well. There are others but these are 3 that I know of.

I think the question of ID's is very subjective depending on the product, organism isolation, location, frequency, etc. I'm a proponent of performing a baseline ID "study" to get an idea of what's present in the beginning of the process and going to a risk-based ID program from there....again, this varies widely based on product/firms.

Assay-Related Culture Passage - Question

Regarding Culture Passages, I have always wondered whether using a purified culture (reference/type or unknown) in an actual growth-related assay step, would be considered as an additional passage e.g. In the case of biochemical ID using substrate-based technology e.g. Biolog / API etc, or antibiotic susceptibility testing (agar / broth).
I have always considered such a step as a passage and aimed to ensure that starting test material for growth-based assays is no more than 4 passages from the reference or stock source, whereas for non-growth-based assays I can use material that has undergone 5 passages. Am I being overly zealous?
I would be interested to get some consensus.

BCC test frequency requirements for non-sterile aqueous products

I need your guidance regarding BCC test frequency. Should we apply batch-wise frequency for BCC test or can be the reduced (skip-lot) testing applied as though non-sterile solid dosage forms (every tenth lot for example) ?

A1: Currently no USP monograph has a requirement for the absence of Bcc. The test would only be applied routinely after an adverse risk assessment to multiple use, aqueous non-sterile drug products as recommended in USP <60>. I understand the FDA is requesting that the test be added to some regulatory submissions but companies would need to assess their marketed products.

I would question the value of the strategy of conducting skip-lot testing on a low risk, compressed tablet. My experience they typically have <10CFU/g. Microbial contamination is best controlled by using pharmaceutical ingredients of good microbiological quality, maintaining your purified water system, and controlling the manufacturing steps with a potential to add microorganisms to the drug product not by periodically testing the product.

Calculate SAL

I would appreciate it if anybody can share his/her expertise on how to calculate Sterility Assurance Level.

Needs more clarification on SLR(Spore log Reduction).

Any reference/method/procedure with details of how to do this would be greatly appreciated.

A1: The formula is as follows assuming utilizing fraction negative values from BI sterility testing. It is referenced in ISO 11135 for EO sterilization and thermal processing as well.

D = U / (Log N0 – Log NU)
Where:
U = exposure time (minutes)
N0 = initial population of the BI
NU= ln(n/r)

n = total number of replicate BI's at U
r = number of BI's sterile at U

As for SAL
SAL = 10^(N0 - SLR)

A2: You can refer to the attached article for SAL calculation.
Sterilization Mathematics_Palash Das.pdf

Container Closure Integrity Testing

I would appreciate it if anybody can explain to me how to perform the container closure test in conditions of overpressure.

A1: please search for "Dye Immersion Test ", which can be used for this parameter.

e.g. Nelson Labs offers this kind of test:
https://www.nelsonlabs.com/testing/container-closure-integrity-dye-immersion-bacterial-immersion/
and explains on its page how it works.

A2: I'm not sure what you mean by conditions of overpressure. I know that there are testing protocols that rely on pressure and vacuum cycles to allow for shipping studies. I believe that ASTM may have a cycle defined, but I can't find the reference to it. I am sure that someone will provide it.

If you can clarify what you are thinking about, this would be great.

A3: In my company, we are submitting a new product, but the FDA is asking if we tested container closure under conditions of overpressure. Here nobody seems to know anything about this requirement.

So, I wrote to the forum, hoping that someone could help us.

A4: I recommend you start with USP <1207〉 PACKAGE INTEGRITY EVALUATION—STERILE PRODUCTS and select the most appropriate leak detection test for your product and justify that to the FDA.

A4: There are different types of container closure integrity test (CCIT) methods that use over pressure of the container to force tracer gas (Laser Headspace) or liquid (Blue Dye Immersion) through a defect. If a defect exists then the tracer gas or liquid will be detected in the vial after the over pressure cycle. These analytical methods (all described in USP chapter <1207>) demonstrate that the container and process to assemble the package create a seal which will prevent any impact to product stability or sterility. As Chris mentioned these methods can also be used to conduct shipping studies to be sure changes in storage conditions during transport does not impact CCI.

Has your team performed any CCI studies on the product container?

A5: Our team perform the microbial ingress testing in the product container (vial + rubber stopper + stopper flip off). So, I was wondering if this type of testing could be performed “under conditions of overpressure”.

A6: What kind of container it is? In the company where I work we use a special equipment to inspect the seal for pouches and plastic cups sealed with foil and Tyvek (Busrt Test) , the equipment use water and it use pressure, for each container a different pressure is used. Also some companies use any solution to dye like Methylene Blue, immerse the container in the solution and apply vacuum (This is used for plastic blisters). I can provide you more information if you think the equipment that I mentioned can work for you.

A7: Our team perform the microbial ingress testing in the product container (vial + rubber stopper + stopper flip off). So, I was wondering if this type of testing could be performed “under conditions of overpressure”.

If you could give more details about the equipment that you use, I will be grateful.

Justifying Higher Device Endotoxin Limit in ST72

ST72 has the following regarding device endotoxin limits:

5.4 Bacterial endotoxin limits shall be determined according to appropriate regulatory requirements and be consistent with intended clinical application.

NOTE 1: For endotoxin limits see current USP <161>, FDA guidance for devices labeled as sterile [51], FDA Q&A guidance [43], etc.

NOTE 2: For ophthalmic products, consideration should be given to the overall risk to patient endotoxin exposure when multiple healthcare products from the same manufacturer will be used together during the same surgical procedure or placement in the same surgical site[49].

NOTE 3: For subcutaneous implantable medical devices without systemic exposure higher endotoxin limits *may *be justifiable, with additional supporting data *depending* on the risk/benefit of the device. (See Cartmell, T., etc. al. [56] where the doses needed to elicit a subcutaneous response was 10 times that of the standard IV dose to produce a similar peak rise in temperature.)

I'm curious to know your thoughts on NOTE 3 regarding "higher endotoxin limits may be justifiable". I've reviewed the (one) reference cited in support of the note, and it's a university study with no references or controls to be able to link or liken the study to the USP rabbit test.

I understand that the historical 20 EU/device limit was set taking into consideration the possibility of 200EU being present on 1 device in a test pool of 10 devices in an extraction volume of 40mL so that the limit is

200EU/400mL (total extraction volume) = 0.5 EU/mL. I also understand that the historical limit is 'high' due to the inefficiency of recovery of endotoxin from devices.

Based on the Cartmell reference in ST72, where "the doses needed to elicit a subcutaneous response was 10 times that of the standard IV dose to produce a similar peak rise in temperature", are we to infer that the endotoxin limit for a subcutaneous medical device may be even up to 200EU/Device, which would translate into 2000 EU/device in a test pool of 10 devices? Have you in your experience observed endotoxin limits higher than 20EU/device for a subcutaneous medical device?

Microbial Contamination Test in Non Sterile Products

I have some doubts regarding microbial contamination tests in non sterile products especially iron folic syrup.

1. We are performing microbial contamination tests in non sterile products routinely in Iron Folic Syrup as per pharmacopoeia. For Total Aerobic Microbial Count, We take a 10 ml sample in 90 ml diluent (stock) and filter 10 ml containing 1 ml by 0.45 micron cellulose nitrate filter paper. During filtration, filter paper is to be choked. What should be followed to eliminate this type of problem?

2. For presence or absence of E.Coli tests per ml, we should filter 10 ml samples from stock and filter paper inoculate in scdm medium for enrichment or can direct inoculate 10 ml samples from stock into SCDM for enrichment. what should be followed directly or by filter paper. Or It depends on what we do follow in TAMC as Pour Plate Method or Filtration method.

3. We do not find any turbidity, gas or acid production in MacConkey Broth after 48 Hours Incubation period also, then we will shift to MacConkey Agar Medium or stop the test and release the test report as Absence of E.coli per ml.

Please clarify all the doubts.

Sanitizing wheels of carts

A topic that I've been discussing since my earliest days in the industry is the science behind the best way to sanitize wheels of carts (such as monitoring carts, pallets). Assuming this is in a facility with a spore problem (significant). The idea would be to mitigate spread while overarching root causes are addressed. Some think spraying wheels with (detergent) is the preferred method, and others feel that physically rolling the wheels over (detergent) soaked pads is the preferred method. What is the opinion of the group?

Regarding detergent... I can't put my finger on why 6% HP seems inadequate to me, to mitigate a large spore problem in the manner described above. My thought is that a much higher concentration (35% ?) would be needed. Fungal spores at 6% I'd think maybe / yes. Bacterial spores, I'm unsure. Any thoughts?

A1: There's two parts to the cleaning process, cleaning and disinfection. Using a detergent to remove any debris is a good idea and generally works better with some physical removal with scrubbing or rubbing. The disinfection/sanitization step is the second step of that process where you disinfect the wheels with HP. There are commercial products like Spor-Klenz that are sporicidal. Those should kill spores that are present, given the correct dwell time. Here's a link to a great paper on sporicides and spores (even if it's old). I'd also reach out to your clean room sanitant supplier as they usually have very good data on killing spores.

A2: I have used ozonated water quite effectively for this purpose. You can either spray it on the wheels or place the ozonated water in a pan and set the wheel in the pan of water.

It is both antimicrobial and sporicidal. Typically it runs at 5-8 ppm.

A3: In addition to cleaning and sanitization, the use of dedicated carts to each classified area and use of conveyor belts and rails that do not involve contact with the floor are good control strategies.

A4: Regarding sanitizing cart wheels, have you seen the carts by Veltek, that eliminate this concern?

A5: Excellent paper on sporicidal agents. I fully agree with your proposition that cleaning prior to disinfection is the best way to eliminate spores (akin to washing your car before waxing it). In fact during the 2017 PMF Fall Forum there was an outstanding presentation showing that cleaning a floor contaminated with spores three times removed almost all of the spores; when a disinfectant was applied afterwards there were no spores remaining.
This is the type of information that is presented at the PMF Forums. For anyone interested we are hosting the annual (remote) Spring PMF Summit (Forum) this year on April 26-27 titled "Environmental Monitoring – Interpretation and Product Impact" (Tim will be a presenter at the Summit). If past PMF summits are any indication, this will be another great one to remember.
Here is a link to the 2017 presentation: https://bit.ly/3cam7zX
Hope to "see" you at this year's summit. Here's a link to it: https://conta.cc/2P0UlgL

A6: There is a company that showcased some really cool carts at PDA Micro conference two years ago. The cart was linked in two sections to prevent transfer contamination. I have not tried them but they looked very impressive. Eliminates needing to wipe down wheels because they do not cross the classification.

See link.

https://sterile.com/product-category/cart-transfer-systems/cart2core-system/cart2core-system-details/

In plants I have worked we tried to avoid moving carts into the next classification, however that requires more people to move the materials.

A7: Those are the carts from Veltek and they are great!

Suitable method for sterility test of lyophilized antibiotic injection

Please suggest me suitable test techniques for sterility test of lyophilized antibiotic products by close canister method.

A1: We have a canister designed to work with antibiotics that also includes a hydration step.

Hand Sanitizer Alcohol Free Product Recall

Today, I noticed that there was a recall notice for an antimicrobial hand sanitizer – alcohol free for the presence of Burkholderia cepacia complex and Ralstonia pickettii contamination. This contamination had been discovered during a routine audit focused on production scale-up during the height of the pandemic. It is obvious that the source of the Burkholderia cepacia complex and Ralstonia pickettii contamination was due to the water system that had been used in manufacturing the affected recall lots of this product.

By doing a deeper dive as to why this formulation had been recalled, you need to look no further than the composition of the product. The active ingredient was Benzalkonium chloride at a concentration of 0.13%. The preservative system consisted of the following components: Diazolidinyl urea, methyl paraben and propyl paraben. In addition, the formulation contains cetrimonium chloride as an inactive ingredient.

In general, Benzalkonium chloride has poor antimicrobial activity against Pseudomonas and Burkholderia bacteria. It is interesting to see that the formulation also contains cetrimonium chloride which is a long-chain quaternary ammonium surfactant that has antimicrobial activity. I believe that cetrimonium chloride is

not in the proposed list of approved active ingredients for usage in OTC healthcare antiseptic products. I wonder if the inclusion of this quaternary ammonium surfactant had increased the potency of the antimicrobial activity of the benzalkonium chloride active ingredient in the formulation. In addition, the usage of Diazolidinyl urea, Methyl paraben and Propyl paraben as a preservative has been well known to have antimicrobial activity issues against Pseudomonas and Burkholderia species.

It would have been interesting to see if there were preservative challenge test results of this formulation against Burkholderia cepacia ATCC 25416. It would also be interesting to see the MIC/MLC test results of the Benzalkonium chloride ingredient with and without Cetrimonium chloride. Finally, it would be interesting to see the ASTM E1174 Standard Test Method for Evaluation of the Effectiveness of Health Care Personnel or Consumer Handwash Formulation with and without the presence of Cetrimonium chloride in this hand sanitizer.

Before being attacked, I will admit that there is nothing being incorrectly done by including antimicrobial boosters such as an antimicrobial quaternary ammonium surfactant in the formulation to increase the potency of the active ingredient in an antimicrobial soap product formulation. I have found that most microbiologists do not understand formulation chemistry to have an understanding as why a formulation fails some way or another. For the most part, we are in our microbial testing silos cut off from the world. For this recalled product formulation, I suspect that there was more than one issue in play to cause this recall besides the contamination source being the water system.

Product impact assessment for product issue

i have an aqueous non-sterile nasal spray, bottle and pump with replaceable cap, 30 day usage period. During filling a stoppage occurred and upon inspection the operator noticed on one of the filled units that the neck of the bottle had a minor deformity and within that there is a pin prick hole. The bottle batch was changed for filling to recommence. Investigation etc ongoing.
My issues is an assessment of the already filled and packed units (approx. 20,000).
When the cap is on the possible hole is covered. They were manufactured in a Grade D environment and covered. In use by a patient they should be re-covered after use.
The thinking is that if there is a hole when it goes to market that the product would end up leaking via the hole as the bottle would not necessarily remain upright and the customer most likely complain and stop using. However, we are looking to assess not having to unpack and manually inspect all filled product and allow the packed portion to be shipped. I need to generate a justification from a microbiological standpoint that even though the product may still be used for the stated period by a customer and if there was pain hole then it is ok for patient use and no impact to product quality or efficacy.

The product is multi use so has preservative. PET data is satisfactory.

What are peoples thoughts on if we can still allow the shipment of this batch with the possible hole and what needs to be included within the justification documented within the deviation investigation.

A1: You never indicated whether your investigation went so far as to inspect the rest of the bottles in the implicated batch or had contacted the supplier to see if similar issues have been reported.

Do not simply rely on PET data or environmental conditions during the fill. If the component does not meet your requirements then I would find it difficult to justify releasing unless you performed a manual inspection of every filled container and performed a risk assessment that it is acceptable to do so.

A2: Without data to demonstrate that this breach does not compromise the product (even non-sterile) you should not ship. Even though the cap covers the "possible hole", as the product is shipped it will experience changes in pressure that expand and contract the containers. It is my experience that the distribution process itself will provide bottles that have leaked upon receipt to the customer, which will contribute to your concern about customer complaints.

A3: Did your company inspect the bottles for the presence of any deformities before they had been used in manufacturing? If the deformity had been detected before use, the bottles should not be used for manufacturing.

In addition, were pin holes present in retained samples of unfilled bottles that had been used to fill product in previous production batches? If so, your supplier has a manufacturing problem in making the bottles for your nasal spray. I understand your reasoning of trying to use PET data to justify the release of the product. However, PET data would not cover the loss of water by evaporation from the formulation over time through the pin hole. You need to determine if this pin hole defect is present in all of the bottles (filled and unfilled).before making any decisions in releasing the filled batch. If the pin hole is not present in all of the bottles, you may need to culled the defective units from the batch.

A4: The investigation is ongoing. The received batch was subjected to inspection and release with no issues identified at that stage. ongoing communication and investigation with the supplier for root cause.

The proposal is to not have manual inspection of the filled and packed units and release the product under an investigation and risk assessment. it is more aimed that, in the unlikely event that there are more damaged units that made their way into the already packed devices that either the customer will complain or we can be satisfied that the quality will be maintained.

The microbiological aspects of potential damaged units which are being asked about how/if we can state that the product is preserved and PET data can support that any additional environmental contamination when it is in market would preserve the products quality.

I myself don't see how we can use the justification of it is preserved so it will basically be ok, but we are being asked to provide a sound summary that can be used to allow its release. I know preservatives are not a substitution for poor manufacturing practices and to ensure initial micro release quality but can it be positioned that it is/will be doing its job when on market?

A5: i think that there may be an appetite to allow the possible complaints if more damaged units are in already filled packs but we do know not that there are even any. As part of the assessment to say that if there were any damaged units that made it safely to a customer without signs of the leaking, could a sound micro justification about the preservative doing its job while it is in use be appropriate and defendable if questioned?

A6: The original batch was inspected and QC released for use with no issues noted.
No more holes have been identified in the devices that were immediately inspected that were undergoing packing, however there is no plan to unpack and insect all filled units. the bottle batch still under filling was cleared and a new batch used to finish the filling process.
The other aspects you mention e.g. evaporation are being assessed by analytical teams but we are being asked to state that the product quality will be ok due to it being preserved.

Im struggling to agree with what QA want to state for this as i know you cant substitute poor manufacturing with a preservative, however when in use it will be doing its job, just not exactly as intended via contamination from patient via the nose spray.

Could it be defendable to rely on the preservative to maintain product quality if the other analytical assessments say it is ok?

A8: Very slippery slope. If you cannot confirm the batch meets its critical quality attributes, including container closure requirements, it should be rejected. PET has nothing to do with this decision. And accepting the notion that there could be damaged product only to be discovered in a customer complaint is a dangerous path to take. Regulators routinely monitor this group. I would seriously reconsider your position.

A9: What is the product (packaging) spec? If it states no leaks, or provides a limit, and it you're above the limit then the product is adulterated and it will be very difficult to justify releasing it, even if all the data look good. This is an example of compliance vs. science.

A10: In reference to your question. I'm a little hesitant in using PET for justification in releasing a batch in which there is a manufacturing issue with the finished product container. Instead, I would feel more comfortable to do a 100% inspection of all filled units to see if there are any more leakers before a decision is ever made concerning release of the batch. From my perspective, environmental contamination will often consist of Bacillus species and other mold species such as Penicillium species. If your challenge testing did not include these additional organisms, I cannot see how you can use your PET results to justify the release of the batch. PET is for determining how well your product formulation will stand up to microbial contamination that is introduced during consumer usage. PET results are not supposed to be used to justify the release of a batch due to a manufacturing issue. For this issue, I'm in agreement with the comments by [names redacted] that this batch should not be released into the marketplace if there is not going to be 100% inspection of filled units for leakers.

A11: In complete agreement with all respondents, the justifications as interpreted are not only unscientific and unsound, but downright high risk (imo).
Quality First - Don't Ship - Quarantine, Complete Investigation - Open CAPA and then use sound Quality / GMP principles and risk adverse thinking to determine disposition.
Two worrying standouts that might highlight concerns with Quality Policy not just release issues pertaining to this particular batch are,
a) The proposed use of active customer complaints to establish risk level associated with non-compliant GMP practice - ("that either the customer will complain or we can be satisfied that the quality will be maintained") heaping hurt upon hurt (double whammy),
b) That your QA group would appear to be even contemplating any such justifications,("I'm struggling to agree with what QA want to state for this")

Going forward and anticipating worst case scenarios (future GMP inspection that reviews this OOS / NCR) you might also need to;
1) Ensure that a strong coherent QA narrative (response & leadership) on this issue is demonstrated and documented throughout your Investigation & CAPA activities, in order to counter perceived inadequacies in policy that might be currently evident,
2) Assess whether such policies have influenced decision making in previous (recent) release / product disposition issues.
Hopefully, QA will step up and support you.

Amphotericin B Bioassay (USP 81)

Can anyone please guide me on how to grow *S.cerevisiae *ATCC 9763 for bioassay? I have tried a lot but not getting a full lawn of growth.

A1: To perform a USP 81 antibiotic assay on Amphotericin B, you need to use Antibiotic Assay Medium # 19 as the inoculum medium to grow S. cerevisiae ATCC 9763. You have no choice in using another microbial growth medium. If your S cerevisiae fails to grow on this medium, I would suggest that you get a different lot of the culture. Since I have no idea in how your store your antibiotic test cultures, there could be something wrong with your current lot of the strain. Furthermore, have you tried to initially grow your strain on ATCC Medium 200 (YM agar or YM Broth) before subculturing it onto Antibiotic Medium #19? ATCC initially recommends this medium to grow this organism from lyophilized vials. Have you also initially used Emmon’s modification of Sabouraud’’s Agar to initially grow this strain? If your culture is not able to grow on any these media’s, there is something definitely with your strain.

A2: Interesting. USP <81> recommends S. cerevisae 9763 on Medium 19 incubated at 29-31 degree C for 48 hours. The original method in Kavanagh's Analytical Microbiology recommends C. albicans with different medium containing malt extract not beef extract incubated at room temperature for 3-4 days.

Poor growth may be attributed to the inoculum quality, media selection, and incubation conditions. Try extending the incubation time to 72 hours.

A3: Another point very important, be carfull with the Agar temperature, When you inoculate the microorganismos, the Agar must be no more than 43 °C because S. cerevisiae ATCC 9763 is so labile, it happened to me once.

MLT and test for specified m/o

Could you recommend me books, articles relating to MLT and test for specified m/o to improve my knowledge.

I'm guided by USP 61, 62,1111 and USP FAQs.
I've read partly the book by Lucia Clontz "Microbial Limit and Bioburden Tests" as well.

A1: I would recommend the following:

Microbiological Quality Assurance and Control – A Practical Guide for Non-Sterile Manufacturing. D. Roesti and M. Goverde (editors) J. Wiley & Sons 2020

Environmental monitoring

I have a question for improve the Time taking the air of the environmental monitoring can i change the quantity of liters taken for less liters for example 1000L to 500L?

What can i do for do it the change?

A1: I assume you are referring to viable sampling.

I am not a fan of taking less than a cubic meter of air, ever. Depending on your sampler, you are sampling for only 5-35 minutes in order to take 1000L of air, which is providing good dynamic data. Why cut that short?

I will step off my soapbox and understand that some firms may decide to go with smaller sample sizes in Grades C and D areas and calculate the results per cubic meter (again, air is not homogenous and you are over/under estimating your counts).

If you decide to do so, it should include a risk assessment and rationale with supporting data to justify the change in sampling volume.

You can make the adjustment from your samplers' user menu (either time or volume, depending on the model).

A2: In general the answer is "no", since we can't extrapolate air contamination as we do in other microbiology tests because air isn't homogenous and each sample is a snapshot. Some firms do this in non-critical areas but that's usually in a much less regulated environment. What grade is your room that you're looking to reduce sampling?

A3: What about the air sampling in non-sterile production? In which you must sample 1000L air for bacteria and 1000L for fungi. And also the same air volume for the compressed air in the same room. What do you do when you have to sample 30-50 rooms? Can you consider the application of passive settle plates method instead of active air sampling method?

A4: Why "must" you sample 1000L individually for both bacteria and fungi? There's no requirement to perform dual sampling at each EM site.

No reason you couldn’t perform an individual sample on TSA at each location with a validated incubation scheme and obtain sufficient bacteria and fungal counts on the same plate.

A5: Avoid excessive environmental monitoring in non-sterile manufacturing areas. Periodic monitoring may be useful depending on the products manufactured.

USP <1113> states:

"In general, environments for tablet and powder- and liquid-filled capsule manufacturing should require no monitoring or infrequent monitoring. Monitoring programs should be risk based, and the frequency and number of sampling sites should reflect the risk level. Manufacturing areas for higher-risk dosage forms such as inhalant products require more frequent monitoring and typically are manufactured in rooms classified to a particulate air quality level, e.g., ISO 8."

"For most non-sterile product manufacturing environments, because of their limited environmental controls and comparatively low product risk, the establishment of alert and action levels may not be required. Environmental monitoring is considered an informational survey of the general hygienic conditions of the environment and should not be used in product-release decisions. Monitoring of unclassified environments is not required”.

A6: For non-sterile product manufacturing there is no requirement to sample 1000 L of air. Depending on the area classification and gowning requirements sampling 1000 L of air may yield too many colonies to count. You can reduce the air volume per sampling site. This along with the other suggestions already provided, use one plate for both bacteria and fungi (this is quite common), and periodic sampling per USP <1115> should reduce your environmental monitoring.

A7: In case I sample less than 1000L air (in non-sterile production) how I will address the results, since in sterile production it is addressed as CFU/m3.

A8: Is grade 8 for oral solids, but i normally have results less than 20 UFC/m3 and we want to do less time in the monitoring.

A9: If you are comfortable to sample 500 lit then multiply the result with 2 factor. Then equal to 1000liters.

A10: With a limit test you typically must sample the amount specified in the limit. For example CFU/g or ml for non-sterile drug testing and CFU/m3 or 1000 mL for environment.

Other than the Grade D requirements in the EU GMP Annex 13 I know of no regulatory requirement for a specific air cleanliness standard for non-sterile products.

A11: an answer most don’t like hearing, but if you need to sample 50 rooms and don’t have the time, you may need to look at expanding resources. It is a basic quality requirement to be adequately staffed such that you’re not prone to considering cutting corners when performing quality tests regarding the health of your facility.

A12: I would like to provide suggestions

1. I would suggest to perform Risk assessment with respect to product risk and reduce the sampling locations instead of performing at 500 litres .
2. Logically sampling at 500 litres and Interpreting by multiplying with 2 sounds ok bt expressing of results at cfu/m3 might be questionable. There is no specific guideline requirement for Non sterile facility ,upcoming WHO guideline for OSD may provide insight in this topic.

A13: You are correct that there is no regulatory standard for air cleanliness in the manufacturing environments for non-sterile products. In general, non-sterile product manufacturing occurs in rooms that are not classified. In the absence of a microbial standard, most people will often start using the microbial air standard of a Class D environment for these areas and establish an internal standard based upon

generated data. It is not common to see the presence of HEPA filters in non-sterile manufacturing environments. Instead, the air filters for a non-sterile manufacturing area have a classification called Merv that has a lower filter particulate retention in comparison to HEPA filters.

Cleanroom Gowning

Does anyone have information on the relative cleanliness of different gowning materials? That is, comparison between the use of technical suits, tech suits with frocks, coveralls over street clothes, etc.? I'm especially interested in the shedding of particulates depending on the type of PPE worn.

A1: I need some more information to best answer. What class clean room are you in? What is the application? When you say frock or coverall, do you mean single use? Tyvek? SMS, Microporous Laminate? Reusable? Integrity 1800? Altessa? Is a tech suit single use or disposable? Cotton/poly? All Poly? There's so many variables to be able to formulate a meaningful response.

A2: The manufacturer will give you the data on the garment. They should follow IEST-RP-CC003.3 or similar method to demonstrate shedding/particulate generation using an apparatus such as an H-Y tumble drum.

If your vender cannot or will not provide that data, pick another vendor.

A3: I may have wrongfully assumed you were referring to the shedding characteristics of the actual garment only. That is where the IEST method would be utilized.

You didn't state reusable or disposable garments of which there are many different considerations.

I will assume (which, clearly, already bit me once), that you are looking for information on PFE (particulate filtration efficacy) and BFE (bacterial filtration efficacy) of the garments (I.e. ability to keep particulate and contamination generated by my operators inside the garments themselves)

As Lara mentioned in her response, each gown (material, reusable/disposable, stitching/assembly, etc) has specific retention capabilities. Naturally, the starting particulate load and bioburden of the operators, immediately prior to gowning, play an important role and may cause you to exceed the capability of the garment to "hold it in"

This is also why maximum allowable wear time should be established based on risk-based appropriate undergarments, activities that will be performed as well as the physical capabilities of the gowns.

PFE and BFE data should be available from the manufacturer.

In short, "it depends".

Germ identification method

Our contract laboratory will switch the germ identification from another system to the MALDI-Biotyper system, and the possibility of germ identification there using PCR (16S-rDNA) should also be eliminated in the future.
We are now checking whether we can switch all identifications to identification via MALDI.

Do you know of any official or guideline requirement if the use of gene-based germ identification (by means of PCR) is mandatory for certain tests / indications, or can a complete switch to MALDI as the primary identification method be implemented (apart from the general suitability for the types of germ, of course)?

Affected tests are: bioburden; sterility testing; microbiological clean room monitoring (class A-D), total microbial count of water monitoring (PW, WFI).

A1: There are no general requirements for the microbial identification system to use, especially when the microbial ID is secondary to the initial recovery of microorganisms. However, if the lab will use MALDI as an alternative to a compendial assay in which you are testing for presence/absence of a particular organism (i.e., USP 62 specified organism test), then they would be expected to perform the necessary validation studies to show the new test is non-inferior to the existing compendial assay.

The only requirement for using a NAAT I am aware of, assuming it is still in place, is to disqualify a failed sterility test for the Australian TGA, where you would prove the organism in the failed sample came from another source (such as personnel).

So, as long as the MALDI has been properly validated (e.g., see USP 1113), it can be used for microbial IDs for microorganisms recovered from bioburden, sterility, EM and water test media.

A2: MALDI TOF mass spectrometry is widely used in both clinical and pharmaceutical microbiology to identify microorganisms by both inhouse and contract microbiology labs. There are many peer-reviewed articles on the accuracy of the method.

A3: When moving to any ID system, the method is only as good as the library behind it.

You should have no issue moving to the MALDI-TOF (proteotypic method). In the instance an ID is unavailable, most system vendors will utilize 16s PCR to provide a final ID (and then hopefully generate spectra to add the ID back to the maldi).

You'll find that proteotypic ID systems are quite good at providing accurate results when looking to associate significant failures (sterility, APS, ISO 5, etc) to probable root causes as they are based on ribosomal proteins (for which the translation and transcription precursors are DNA).

I'll remind you that most "concern" lies with the old adage that "genotypic ID systems are recommended", which came from the 2004 FDA aseptic guidance document. This was long before MALDI systems were available for ID.

From a regulatory standpoint, you should be just fine going with your new method.

A4: I can understand as to why your contract laboratory is going to implement the usage of MALDI-TOF for conducting identifications of isolates. However, it is my understanding that MALDI-TOF library for mold needs improvement based upon several scientific published journal articles. Normally, the usage of gene sequencing is considered to be the gold standard for conducting identification of isolates by sequencing the 16s rRNA for bacterial identification and the ITS2 or D2 of the LSU of the rDNA for the identification of fungi. I do not see an issue if both systems are operational in a laboratory to do identifications as long the IQ, OQ and PQ portions of the validation for the instrument had been performed and you have properly trained personnel to operate each piece of equipment. If an isolate is not in the MALDI-TOF library, I have seen companies perform gene sequencing of the isolate to determine an identification and add the proteomic pattern from the MALDI-TOF as a supplement to their in-house library.

As far as a requirement that a certain or particularly identification system that has to be used for the identification of isolates obtained from conducting microbial testing in a pharmaceutical environment, I have never see one. I have seen many companies use both phenotypic (e.g. biochemical identification kits/cards) and genotypic/phylogenetic identification methods in a lab as long each of them have been validated.

A5: In switching to Maldi-Tof it is important to know the limitations of the system, e.g., there are some closely related organisms that cannot be separately identified. This can result in the inability to identify some organisms. I recently had a client that just accepted these organisms as non-identifiable.

Unfortunately, one of the potential organisms was extremely pathogenic and it was a non-sterile product. You need to have a system for handling non-identifiable organisms.

A6: Every identification method has its limitation. No single identification method is absolute. The MALDI-TOF is a complimentary method.
PCR can be use when dealing non-identifiable microorganism by MALDI TOF. Other method such as fatty acid profile can be used as well.
I am not sure about eliminating any identification method in the future. Non-identifiable is pending on the method used.

A7: I agree that the databases are the most important aspect of whether or not you're comfortable making this change. In the "early" days of some microbial ID systems, you could count on Staph hominis never showing up...because it wasn't in the database! Times have changed quite a bit but Marc is right in that the 2004 FDA Guide for Aseptic Processing mentions that for investigations into critical processes/failures, " Genotypic methods have been shown to be more accurate and precise than traditional biochemical and phenotypic techniques. These methods are especially valuable for investigations into failures (e.g., sterility test; media fill contamination). However, appropriate biochemical and phenotypic methods can be used for the routine identification of isolates."

I don't think you'd have a problem using MALDI if you feel the databases are comparable. Also, as stated in previous replies, having a genotypic method at your disposal would be very useful to fill the gaps in any database. Finally, as a contract lab, you may want to consider what your customers want and if they'll accept a proteotypic ID vs a genotypic ID for a critical investigation.

A8: Why are we quoting the 2004 FDA Aseptic Processing Guidance when MALDI TOF mass spectrometry for routine microbial identification was not available 16 years ago.

The FDA should update the guidance or withdraw it and defer to the revised EU GMP Annex 1.

A9: I was giving context to Marc's comment so that people knew where it was coming from. Yes...it's quite outdated but still used!

A10: In my line of work, I have maintained sometimes a different approach to microbial identification systems because a lot depends on the type of manufacturing and risks for likely contaminants.

For example, although the 2004 FDA guidance exalts genotypic ID over phenotypic ID for certain aspects, that assumes the most likely contaminants from aseptic processed products are human and environmental organisms. Therefore, biochemical ID systems, which had there basis in the clinical world, did not have the coverage or database for environmentals.

But what if the most likely and actual observed product contaminants or sterility failures are from clinical based raw material (i.e. cell collection)? In that case, a clinically founded phenotypic system can have great accuracy and ability to sufficiently ID a bug that originated from clinical source.

My point is, just because "Genotypic methods have been shown to be more accurate and precise than traditional biochemical and phenotypic techniques" does not mean a phenotypic system will not be sufficient and very accurate for certain applications.

A11: Let me add my comments to this discussion. When considering what identification system to use I think it is important to remember the rapid changes that are occurring regarding microbial taxonomy. For those with access too or remember Bergey's Manual 7th and 8th any Gram stain positive rod shaped cell, that was aerobic or facultative and formed a bacterial endospore was placed in the genus Bacillus. Following Carl Woese's pioneering work with the 16S rRNA, a paper was published in 1991 (Letters in Applied Microbiology 13:202) which showed the phylogenetic heterogeneity of the members of the genus Bacillus. Since that work the members of the genus Bacillus have been assigned to many new genera.based upon phylogenetic analysis and/or chemotaxonomic characteristics. More recently there is a proposal to limit the genus Bacillus to members of the subtilis and cereus clades (IJSEM 70:406 and

IJSEM 70:5753). At the present time the gold standard for identifying bacteria is the 16S rRNA sequence. To determine if you had a novel bacterial species the 16S sequence cut off was initially proposed to be 97%. That value was changed to 98.7%. In 2015 a publication in IJSEM (65:1929) reported that one threshold value does not universally apply. It should be noted that even if the 16S sequence similarity is greater than the threshold value it does not mean that two strains are the same species. This reflects the difficulty 16S sequencing can have in always resolving at the species level. We published a novel species that had a 16S sequence similarity of 99.8 % to its most closely related species (IJSEM 69:1376); we used MLST, DDH and ANI to show that we had a novel species. Now that the cost of sequencing is so cheap microbial taxonomy is now based on the genomic sequence (Methods in Microbiology, vol 41:1; William Whitman). Even with the requirement of genomic sequencing 1000s of novel species are being proposed on an annual basis.

To summarize, whatever system that one uses it is important to remember that microbial taxonomy is very dynamic and constantly changing and the best system(s) in my opinion, is/are the one(s) that can accommodate or reflect this.

A12: From my time in this industry I think microbial ID's has been one of the hottest topics that always elicits an opinion! Yes, Tim, I agree, I wouldn't discount any ID system and the databases are growing all the time. It seems people have VERY strong opinions on what they like.

Suitability of counting method in the presence of the product

Is the suitability of counting method in the presence of the product as per 2.6.16 Eu. P mandatory or not. if yes at witch stage shall we conducted it. Case of new products in generic oral dosage form.

WFI Endotoxin Hold Times

I was wondering if anyone had any literature on hold times at 2-8C for WFI.

A1: You should find some useful information in PDA TR #82.

A2: I was going to ask this very question, but also, does anyone know of literature on hold times for in-process prepared buffers and solutions in general? E.g. PBS, HEPES, CaCl2, MgCl2, as some examples.

A3: We have established hold time for Inprocess testing with Bioburden and Endotoxin parameters.

Limits for Bioburden and Endotoxin has been calculated by back calculating with specification limits and scientific rationale.

A4: From my experience, there is a somewhat simple, but not particularly satisfying answer: preferably, study the hold time for each solution yourself!

The good news (depending on your expectation): At least in pure water, 24h can easily be reached without critical impact on endotoxin activity.

Why study it yourself?
While lipopolysaccharides themselves are rather stable molecules (see dry heat depyrogenation >180°C), the activity of endotoxin can be modulated quite a lot, see Low Endotoxin Recovery (LER).
Potential parameters include:
- Container
  - Material
  - Leftover cleaning agent

- Solution

- all ingredients and their interaction, i.e. not necessarily the sum of the parts (stimulation or neutralisation or ...)

- Endotoxin preparation
  - Bacterial strain
  - Culture conditions
  - Endotoxin purification method
  - Formulation

- Storage Conditions
  - Temperature
  - Light
  - Agitation

That is not to say that all of these factors were equally critical, but they may reduce the conclusions you can draw from literature on your own storage conditions. I would recommend simulating them as closely as possible and feasible in your QC lab. Furthermore, Reference or, alternatively, Control Standard Endotoxin (RSE, CSE) are the spikes of choice.

A5: Apologies, I should have looked at the original subject and PDA 82. My focus for now is bioburden, not endotoxin. I'm interested in any literature regarding hold times for solutions, such as those listed below, before testing bioburden.

Consequently, as for endotoxin, in my previous job I did in fact run hold studies on purified water held at 2 – 8C for up to 7 days, 0.25 EU/ml spike. No issues found.

A6: I think [name redacted] gave a good response to this question. Each item to be held needs a hold time study done by the tester. There are so many variables to be considered as Thomas so nicely stated. Even WIFI is different from company to company. I once ran into a sample of WIFI from a company that was inhibitory to the assay. Unfortunately this is a case where you will need to do the studies under your conditions for the studies to have any value to you.

USP Sterility Test Incubation Period

In a conversation that a client had with me, they were asking me questions about for the 14-day incubation period for the USP Sterility Test for products that do not have a short shelf-life such as positron emission tomographic (PET) products, cell, and gene therapies, and compounded sterile preparations (CSPs) prepared for immediate use. They well understood that rapid microbial methods have been recognized for a while as a potential alternative to 14-day USP<71> Sterility Test since 21 CFR 610.12 had been revised in 2012. At one time a long time ago, the USP Sterility test by membrane filtration had a 7-day incubation period. However, it was my understanding that the incubation period for USP Sterility Test had been increased from 7 to 14-days in 1998 because of concerns in having contamination with slow-growing organisms such as Cutibacterium acnes. In addition, aseptic environmental organisms could be damaged or in an injured form so that it requires a long time for recovery of these organisms in a microbial growth medium.

The question that they had asked me was there ever a published scientific peer review journal article showing that the increased incubation time to 14-days for a USP Sterility Test was able to indeed detect the presence of slow-growing organisms or injured organisms in parenteral products during the additional incubation period of 7-days to a total of 14-days. They were wondering if this increased incubation time to 14-days was ever proven scientifically or is just based upon a so-called urban legend that the usage of a longer incubation time would detect the presence of these slow-growing or injured organisms in a sterile parenteral product.

I told them that I was only aware of one journal article that the increased incubation time to 14-days had been justified (Bugno A, Pinto Tde J. Incubation time in sterility tests for pharmaceutical products. Boll

Chim Farm. 2002 Nov-Dec;141(6):453-6. PMID: 12577516). However, this article was published after the incubation period had been changed from 7 to 14-days. In addition, I believe that this study had used ATCC strains as the inoculums. Is anyone else aware of published scientific articles for justifying the 14-day USP Sterility Test before the change had been implemented? Is there an article showing that there was an increase in the detection of microbial contamination in parenteral products with wild-type slow-growing or injured organisms between 7 and 14-days of the increased incubation period for the USP Sterility Test?

A1: I remember references from TGA Australia, specifically with membrane filtration method, where certain organisms required longer than 7 days. If I recall, membrane filtration had 7 days incubation while direct transfer was already 14 days. The Australia paper led to membrane filtration method increased from 7 to 14 days

I don't recall the actual reference paper but I am sure can be found, or other PMF and USP folks will know.

A2: I think it would be hard to find this kind of data unless it was an actual FDA recall. Customers who don't release product based on sterility failures certainly wouldn't advertise these failures and unless obligated by the FDA to recall, we'd never know if they had sterility failures or not. I can tell you that customers do have sterility failures and C. acnes is a concern because it *can* grow slowly, though not always in the classical sterility scheme. There are many organisms beyond C. acnes that are slow growers that can also show up past 7 days.

A3: Key articles may be Bathgate et al 1993 The incubation period in sterility testing PDA J. Parent. Sci. & Technol. 47(5): 254-257 and Besajew 1992 The importance of the incubation time in the test for sterility Pharm. Ind. 54(6): 539-542

A4: [name redact4ed] cites Bathgate et al (1993), in this recent APR article:
http://www.americanpharmaceuticalreview.com/Featured-Articles/574806-Live-Stressed-and-Dead-Microorganisms-Their-Role-in-Microbial-Test-Method-Validation/

Microbial contamination

I have some doubts regarding microbial contamination tests in non sterile products.

1. For presence or absence of E.Coli tests per ml, we should filter 10 ml samples from stock and filter paper inoculate in scdm medium for enrichment or can direct inoculate 10 ml samples from stock into SCDM for enrichment. what should be followed directly or by filter paper. Or It depends on what we do follow in TAMC as Pour Plate Method or Filtration method.
2. We do not find any turbidity, gas or acid production in MacConkey Broth after 48 Hours Incubation period also, then we will shift to MacConkey Agar Medium or stop the test and release the test report as Absence of E.coli per ml.

Please clarify all the doubts.

A1: Regarding question 1, what you will follow (Pour Plate Method or Filtration method) depends on your validated procedure. Specifically, with which procedure you have gained acceptable recovery based on EP 2.6.12 and 2.613. In most cases is the plate count method.

Regarding question 2, even if you do not find any turbidity, gas or acid production in MacConkey Broth after 48 Hours Incubation period, you must subculture to MacConkey Agar Medium and incubate at 30-35° for 72 hours. Then you can release the test report as Absence of E.coli per mL.

A2: Question No:01 USP states "1g " of product to be examined for testing purpose , so it completely depends on firm for selection of methods to suffice the requirement. It's not mandatory to be followed technique which was adopted in Quantitative estimation. It's completely based on your Method suitability.

Question No:02 As per USP there is no requirement for observing results after enrichment in MCB broth, please refer statement" shake the container , transfer 1 ml of soyabean casein digest broth to 100ml of MCB and incubate at 42 to 44 degree Celsius for 24 to 48 hours . Subculture on plate of MCA at 30 to 35 degree Celsius for 18 to 72 hours" So its mandatory to continue your testing to MCA agar. If you get any growth on MCA it should be subjected for identification and confirm the absence of E.Coli.

ISO 5 sampling in ISO 7 environment?

I have come up against something I have not seen before. The individual responsible for passing items into the BSC/receiving items from the BSC during operations (I would call them a material handler) performs self-sampling one time: after operations have concluded, in the ISO 7 environment, on their fingers and wrists. These samples are then held to an ISO 5 standard. The material handler performs no manipulations in the BSC and never enters the BSC past the wrist- again, solely to pass items in and receive items out. They also undergo ISO 7 personnel monitoring prior to exiting the suite.

My questions are these: 1) Is it ever appropriate to perform 'ISO 5' sampling in an ISO 7 environment? 2) Would you consider this individual as having "entered" the BSC and become an ISO 5 operator, requiring monitoring?

Non-Sterile cap for capping of sterile vials

I am trying to find any references or guidance where, any recommendations regarding aseptic processing capping process provided.

One of our client suggesting to use non-sterile cap for capping process because capping is carried out under non-sterile are.

In my understanding for aseptic processing where the final filled and sealed containers do not go under terminal sterilization, stoppered-filled vials that are sterile should be kept under grade A (under aseptic condition) until fully sealed. The sterile stoppered vials may be compromised, if they come in closed contact with non-sterile caps or environment.

Any suggestion to if the suggestion from our client would be acceptable to FDA?

A1: EU GMPs Annex 1 states: Vial capping can be undertaken as an aseptic process using sterilised caps or as a clean process outside the aseptic core. Where this latter approach is adopted, vials should be protected by Grade A conditions up to the point of leaving the aseptic processing area, and thereafter stoppered vials should be protected with a Grade A air supply until the cap has been crimped.

Soybean-Casein Digest Broth/Tryptic Soy Broth with polysorbate and lecithin

I am a QC Microbiologist in a nonsterile manufacturing facility. We generally prepare our broths for USP <61> <62> testing from dehydrated culture media and steam sterilize the batches per a validated method in our autoclave. We are currently working on a contingency plan for scheduled down time of our autoclaves and I was hoping for some guidance regarding ready to use media, specifically Soybean-Casein Digest Broth (SCDB)/Tryptic Soy Broth (TSB) with neutralizers (polysorbate and lecithin).

We currently prepare TSB per USP <62> and add polysorbate 20 and lecithin (TSBPL) prior to sterilization. There are several media manufacturers that offer TSB per the USP formula; however, we are having difficulty finding ready to use TSBPL per our validated formulation. We are considering purchasing the ready to use TSB and adding the polysorbate 20 and lecithin, but were unable to find sterilized polysorbate or lecithin, other than in solution, which would alter the TSB formula.

Does anyone have a source or suggestion for ready to use TSBPL (TSB with polysorbate 20 and lecithin) or, alternatively, a source or suggestion for sterilized polysorbate and lecithin? We have found very few ready to use TSBPL manufacturers regardless of the neutralizer concentration or type (polysorbate 20 or 80), and the available volumes are limited. Any help would be greatly appreciated!

A1: I believe that there are some contract media providers that will provide customized microbial media formulations. Have you investigated this approach? I have done this before by having a contract media provider to provide me with TSB with 4% Tween 20 and 0.5% Soy Lecithin in 500-ml screw-capped bottles. They also supplied a COA with pH, sterility and growth promotion test results for each lot provided.

Unfortunately, it is against the rules of this group to recommend specific media manufacturers.

A2: I agree with [name redacted]. There are vendors that will make custom formulations for you.

If you are interested in making it yourself, you can buy dehydrated TSB and calculate how much Tween 20 and Lecithin you need to add. Add it just like the powdered TSB and mix it. Then dispense it into bottles and autoclave it as recommended by the TSB manufacturer. You will want to perform test that the media will not harm you microorganisms and that it is sterile. I can tell you that it more than likely will not harm your microbes, but having the data for audits is desirable.

Also, the Tween will settle out during the autoclave cycle. When the media has cooled enough to safely handle, you'll want to give each bottle a shake (after tightening the lid of course).

Rotation of Phenolic Disinfectants

I wanted to know what the industry opinion/practice is on the rotation of phenolic disinfectants (specifically LpH and Vesphene). At my facility, we currently rotate between using LpH and Vesphene on a weekly basis. These disinfectants are used daily in conjunction with a bleach solution and 70% IPA.

Is a rotation schedule like this common? Is it necessary? I would like to eliminate the use of LpH if possible, the new LpH III has limitations in preparation that make its continued use at my facility difficult.

A1: The concept of the rotation of disinfectants is largely discredited amongst knowledgeable microbiologists.

USP <1072> states:

MICROBIAL RESISTANCE TO DISINFECTANTS The development of microbial resistance to antibiotics is a well-described phenomenon. The development of microbial resistance to disinfectants is less likely to occur at significant levels, as disinfectants are more powerful biocidal agents than antibiotics. In addition, they are normally applied in high concentrations against low populations of microorganisms usually not growing actively, so the selective pressure for the development of resistance is less profound. However, the most frequently isolated microorganisms from an environmental monitoring program may be periodically subjected to use-dilution testing with the agents used in the disinfection program to confirm their susceptibility, as there are real differences among different species in resistance to the lethal effects of different sanitizers.

The rotation of an effective disinfectant with a sporicide is encouraged. It is prudent to augment the daily use of a bactericidal disinfectant with weekly (or monthly) use of a sporicidal agent. The daily application of sporicidal agents is not generally favored because of their tendency to corrode equipment and because of the potential safety issues with chronic operator exposure. Other disinfection rotation schemes may be supported on the basis of a review of the historical environmental monitoring data. Disinfectants applied on potential product contact surfaces are typically removed with 70% alcohol wipes. The removal of residual disinfectants should be monitored for effectiveness as a precaution against the possibility of product contamination.

A2: Although a few organizations (eg Annex 1..maybe) still recommend rotation among daily (non-sporicidal) disinfectants, most recognize that the risk of microbial resistance to disinfectants is so low that it is not necessary or beneficial. It is recommended that a disinfection program should include a daily (non-sporicidal disinfectant) with periodic use of a sporicidal agent. The frequency of sporicidal can vary from weekly to monthly and should be tuned based on results of environmental monitoring.

If you are under Annex 1, consider this info from an article last year in CT. Many would say the guidance on rotation of two non-sporicidals is still not as clear as it should be and can be interpreted that Annex 1 still has a "should" for that type of rotation.

A3: No, I don't see a need to rotate disinfectants with the same mode of action. Most firms would rotate in a sporicide if anything. Not to say it's wrong, it's just unnecessary.

A4: The current trend in the industry in the past few years is to rotate either one disinfectant and a sporicide or two disinfectants and a sporicide. Therefore, utilizing one phenolic disinfectant and a sporicide to address fungal and bacterial spores would be an acceptable rotation program in the cleanroom.

I recommend the Japanese Pharmacopeia, FDA's Aseptic Processing Guide, PDA Technical Report #70, USP 43 <1072>, Annex 1 draft v12, and several recent industry articles to support your rotation practices.

A5: As others have said, the original reason for rotation (ie preventing the development of microbial resistance to disinfectants) has largely been abandoned.

But by using a sporicide, alcohol and a single non oxidizing biocide you are rotating.

Rotation has some practical benefits though. You don’t have to rotate in the same time frame necessarily eg week about or month about. You could have one week of sporicide and three weeks of non oxidizer eg phenol in your case. So if one product is very corrosive but very effective you could use it for shorter periods to minimize the corrosion. Similarly if one product leaves high levels of residue, presents H&S issues or Is very expensive, use rotation to minimise those issues by using the problem product for shorter periods. Providing of course your modified regime is not inferior to your previous one.

A6: I was going to chime into this threat. It looks like [names redacted] beat me to it. I agree with everything they said. The PDA does have a good technical report (PDA TR 70).

Qualification of steam sterilizer

Need to clarify followings questions regarding steam sterilizer qualification:
1. What statistical approach need to be considered while determining the cold and hot spot in empty chamber heat distribution study?
2. What is the significance of small load or full load thermometric test as per EN285 instead of using routine load in qualification?
3. What is the significance of test pack instead of using Bowie Dick test kit?
4. How to achieve equilibrium time of 30 seconds in the sensor placed within a screw capped bottle or capsule filter or silicon tube in qualification study of porous load sterilization?
5. What all measures need to be taken to reduce the delayed time in qualification study of agar media load sterilization?

A1: 1. What statistical approach needs to be considered while determining the cold and hot spot in empty chamber heat distribution study?

I did co-author a publication that used Response Surface Methodology to locate hot and cold spots in autoclave chambers and set the operating parameters to achieve a 95% probability sterility assurance at the cold spot but the engineering community never warmed up to this approach.

Murphy, T.D. Hokes, J. Green, T.C. *Cundell*, A. M. Application of response surface methodology to sterilization process development and validation of a water cascade autoclave. *PDA Journal of Pharmaceutical Science & Technology. 53(2): p 83-94. 1999*

5. What all measures need to be taken to reduce the delayed time in qualification study of agar media load sterilization?

I am not sure what delay you are referring to.

Typically a cycle of 15 minutes at 125 degree C is recommended for media sterilization. Media should be mixed on a stirring hot plate bringing the media to the boil and transferring it to the autoclave. Depending on the container size and load it may take a few minutes to come up to temperature, and the media will be exposed to additional heat in the slow exhaust cycle.

Remember a chronic damage to media is overheating, reducing the growth promotion capabilities of the media, not achieving a sterility assurance level.

A2: All of these questions and more are clearly examined inPDA technical report 1 revised.

Disinfectant validation 2

I have query regarding disinfectant validation..as per USP chapter 1072 recommends to challenge penicillium crysogenisis spp, is it required to challenge in all facilities or else we need challenge wr we will manufacturing betalactum products?

A1: USP <1072> is a general informational chapter and is not a requirement. *P. chrysogenum is listed as a typical environmental isolate that could be used as a challenge organism..*

Testing pharmaceutical water or products for odor

Is anyone aware of any pharmacopoeia requiring the testing of Purified water or pharmaceutical products for odor?

A1: Some pharmacopeia (Brazil, India, for example) do have a DESCRIPTION section, and they use words like colorless, odorless, tasteless.

Some persons have asked me this, and they have interpreted this to mean "I got to test for color, odor, taste... it is in the monograph", either because a regulator asked them or they were being proactive. Hopefully, no human is being asked to do a taste or odor test. If asked, hopefully the human is smart enough to REJECT the execution of this test. Hopefully, this does not need to be rationalized.

Someone could make the argument that you could test "colorless" by arbitrary human eye or spectrophotometrically. But if you pass a conductivity and TOC test, you would pass a "colorless" test. If a water sample has "color", it will fail a conductivity or TOC test, or both.

Personally, when you read this in the BP, for example, this "odorless" section is written in the DESCRIPTION portion of the monograph - it is not written into the sections on PURITY TESTS or BIOLOGIAL SAFTEY TESTS. I think safety and common sense tells you that says an "odorless" test should NEVER be done.

A2: In the past organic chemists and microbiologist used odor to identify chemicals like esters and bacteria but because of subjectivity and safety concerns with approach it was eliminated.

A3: Refer to USP 1231 for purified water testing.

A4: I'm not sure I would call them requirements but organoleptic testing/observation is at least mentioned several times in the USP. Aside from what I mention below there are many specific monographs that mention changes in odor. These are generally related to raw material testing.

<1168> Section 7, related to compounding - "These tests address individual dosage unit weights (including the average), total preparation weight, pH, and physical attributes such as appearance, taste, and smell."

<2750>, related to dietary supplements - "At least one test should be conducted to verify the identity of each raw material .....may include any appropriate test with established sufficient specificity to determine identity, including chemical and laboratory tests, gross organoleptic analysis, microscopic identification, or analysis of constituent markers."

<1191>, related to stability of liquid products - "Liquid dosage forms.....Instability may be indicated by cloudiness or precipitation in a solution, breaking of an emulsion, nonresuspendable caking of a suspension, or organoleptic changes."

<563>, related to ID testing for articles of botanical origin - "The botanical identification of raw plant materials used in the manufacture of pharmaceuticals.....may also include the inspection of organoleptic features of the botanical tissue such as the presence or absence of a characteristic odor."

A5: I would like to ask a question. Why would you conduct an odor test on Purified Water or finished products? Some non-sterile products contain fragrances and be subject to a so called smell test to verify that a fragrance had been added to the formulation. If a product does not contain a fragrance, I do not see a reason to conduct an odor test. If a formulation has an odor, it is common to using a masking agent to cover up the odor and this occurs during formulation development to hide product malodors.

Instead of looking at USP section 1231 which is a guidance chapter, I would suggest that you look at the USP Purified Water monograph and I believe that there is no odor test indicated.

A6: e.g. within the "German Drinking Water Degree" organoleptical tests (visual inspection, odor & taste) are defined. As this quality of water is defined as feed water for Purified Water (PW) I do not see the point, to not check the purified water for organoleptical parameters. As you already mentioned, fragrances can be subject of the "smell test" and these are obviously impurities in PW.

But to be honest, we do not perform organoleptical tests on our purified water except visual inspection (clear, colorless liquid) as mentioned by Ph.Eur..

A7: I guess the broader question is this: What is the expectation when a monograph lists characteristics such as odor (Odorless) or appearance (Clear, colorless)? Typically, there are no tests provided to confirm the characteristics are met. Is there an expectation that Quality Control provides some manner of confirmation that the product meets these characteristics?

A8: Typically if the pharmacopeia do not have guidance on specific parameters of water, you could easily default to one of the tests listed in publications such as Standard Methods for the Examination of Water and Wastewater or the WHO Guidance on Drinking Water Tests or standard setting groups such as ASTM (ASTM D1292 - 15 Standard Test Method for Odor in Water) or AOAC. At least this way you will actually have a valid reference for the test.

A9: I've also seen this requirement in the Japanese pharmacopoeia - and needing to justify not performing it.

Regarding Millli Q water

We use milli q water for microbiological analysis of drugs as media preparation, buffer preparation etc.

What is the total aerobic viable count limit of milli q water and which specified pathogen should be test?

Generally E. Coli, Salmonella, Pseudomonas and Staphylococcus.
What is the criteria of pathogen absent
E.g
E. coli absent per ml or 100 ml.
Salmonella absent per ml or 10 ml or 100 ml?

A1`: In regards to microbial test specifications for water that had been generated by a Milli Q system, the end product that is produced is Purified Water. As far as microbial count, this water should be in compliance with the USP Purified Water count specification of less than 100 CFU/ml. In regards to testing for the presence of E. coli, Salmonella, and S. aureus, it is highly unlikely that these species would not be present in this water due to the low nutrient conditions that are present in this water. If S. aureus is present, it is in all probability due to a sampling error from shedding from the person taking the sample. It is more likely that Gram-negative bacteria such as Pseudomonas species and Pseudomonas related species (e.g., Burkholderia cepacia complex, Ralstonia, Stenotrophomonas and others) may be present in this water if you do not have a storage tank that has a UV lamp present inside of it for the Milli Q system. I have found this UV lamp inside this storage tank to be very effective in controlling the microbial bioburden of the system. Coliform bacteria testing may be performed if the source water for your system is from a well that is not in compliance with drinking water standards for potable water. From my perspective, I would recommend that any recovered isolates on the plate count of this water be identified to the genus/ species level.

A2: The objectionable organisms limit should be reported per mL, Total count if you use 100 mL filtration should be per 100 mL. For water testing the objectionable organisms are generally B.cepacia, Coliform and pseudomonas species. I have not seen any water tested for S.aureus or Salmonella.

A3: The expectation is microbiological media would be made using purified water, USP.

TSA + tween lecithin plates

We currently use TSA and SDA plates with Tween and Lecithin for environmental monitoring by settling and active methods in controlled rooms. An auditor claimed that the use of plates with tween and lecithin is not common practice. Is this true?

A1: You’re auditor is partially correct.

Media with neutralizers is used for surface sampling where you may have instances of disinfectant residues. It is not needed for air samples (active or passive) nor is SDA of any variety, but that is another story.

To keep it simple, stick with contact plates with neutralizers and standard size (90 or 100mm) plates without.

A2: The addition of neutralizing substances to the media should be justified by evidence of the need to neutralize (for example if they are used in contact plates or swab liquid to neutralize residues of disinfectant previously used in cleaning operations).
They should not be used where there is no evidence that they need to neutralize.
The neutralizing complex (type and concentration) should be adjusted according to the active ingredients of the disinfectants (for example, if sodium thiosulfite based on sodium hypochlorite may be useful).
It would be necessary not only to verify the effectiveness of the neutralizers added to the soil against the disinfectant agent to be inactivated, but also of the neutralizer against any intrinsic toxicity towards microorganisms.

A3: For active air sampling and passive air settling plates, it is not common to use tween and lecithin in TSA or SDA plates. For contact surface monitoring, it is common to tween and lecithin in the diluents for swabs and contact plates such as RODAC or in agar films to neutralizing chemical disinfectant residues.

Preservative Neutralization Validation

I had a question from a client, on how tight is the recovery recommendation 100cells vs 200 cell recovery in performing the neutralization efficacy study for a disinfectant.
The protocol recommends using up to 10^2 to 10^3 log cells per mL for validating the neutralization efficacy of a neutralizer. Typically 10 to 100 cells in 0.1 mL is to be expected to recover after neutralization. My question is how tight is that expected number. What if one gets 200 cells as opposed 100 cells. 200 cells is still a countable number on a plate without diluting the neutralizer/biocide solution. Will it invalidate the study if one gets a 2x number. It is still within the same 2 log.

Appreciate your experiences and comments.

USP 61 suitability

We have one complex formulation which requires treatment with Triton x to break the liposome before starting the suitability process. Once we treat the sample with TritonX, we are not getting Bacillus subtilis recovery. We filter 100 ml and washing it 10 *100ml with Letheen broth (Inoculating the last rinse with <100 CFU).

Just because the above method was not giving us Bacillus recovery, we tried using modified letheen broth and it is working.

Question: Modified letheen broth CoA says, it is used for Cosmetic products to* neutralize Quaternary ammonium. * Should I use this broth for a pharmaceutical product that is treated with a surfactant like triton X ?

If no, then what should we try next as triton x is interfering with bacillus recovery ?

A1: Triton x-100 is a non-ionic surfactant and it is very possible that it is interfering in the recovery of Bacillus subtitles because it is well known that some surfactants have antimicrobial activity against Gram-positive organisms. Have you tried adding EDTA at a concentration of 1 to 5 nM to inactivate the antimicrobial activity of Triton X-100 in your present neutralizing diluent? I have seen some scientific publications indicating that EDTA can interfere with the activity of Triton X-100.

In Modified Letheen Broth, there are no surfactants present. However, Lecithin, Polysorbate 80 and Sodium bisulfite are present in this broth as neutralizing agents of compounds that have antimicrobial activity (e.g., quaternary ammonium compounds, phenolic compounds, formalin, hexachlorophene and ethanol).

I believe that you are able to have the recovery of Bacillus substitus by using Modified Letheen Broth because Polysorbate 80 is able to break up the liposome and there are no surfactants that are present in this microbial broth.

Whatever diluent that you are using for suitability testing, you need to have microbial recovery data supporting your decision and have a full understanding as to why it is working as a neutralizing diluent. Just because the media manufacturer says that it is used for the analysis of cosmetics doesn’t mean that you cannot use it as long as you have adequate suitability test results on file.

Over incubation of selective media due to holidays

I want to get some insight on what the industry practice is for incubation of selective media plates.

For example:

MSA/CAB- incubation period is 18-72 hours-on completion of 72 hrs, if it is weekend (Saturday/Sunday), the plates are observed on Monday morning. Is it a deviation as USP doesn't say anything for this type of over-incubation. We do not remove plates till the completion of the maximum incubation period if there is no growth. In case of XLDA-18-48 hours, if on 48 hours it is a weekend, then it is observed on Monday.

All the media is having positive and negative control along with the incubated sample to mitigate the microbiological risk.

A1: You can apply a validation study and prove that in more than 72 hours the growth is not affected. Then you can extend your incubation during holidays.

Water used in analytical chemistry labs

Where analytical labs have bench top water units (e.g. Elga) for generating purified water for their use e.g. reagent and sample prep, does this water need to meet all of the requirements of purified water. For example must I sample and test these units for microbial quality (USP/Pharm.Eur) and Nitrates (Pharm.Eur)?

We currently monitor these units with a monthly sample for microbial TVC, conductivity and TOC and have a 3 monthly sample for Nitrates.

I'm wondering if we can remove nitrates as not applicable for these systems? incoming water quality by local authority is reviewed on a monthly basis.
I don't see the microbial quality attribute as being necessary for the waters intended use and want to remove this.

A1: For water quality Type I and Type II micro test is necessary

A2: In USP General Notices Section 8.230.30 (Water in a Compendial Procedure), it clearly states that Purified Water shall be used unless otherwise specified.

Also, in USP 1231, it says (in multiple locations) to use water of a suitable quality for its intended purpose.

Consequently, this allows you to test for impurities that would be relevant to the compendial tests you do in the lab. In USP, if the water is used for ICP-MS, you could probably make an argument that microbial load is not impactful.

I do not know the EP details as well, but I would expect that there is a similar verbage that allows one to use water of suitable quality for its intended purpose - meaning you only need to prove that the relaxed specs are acceptable for your lab tests.

Water conductivity EP 10.0

In European Pharmacopoeia purified water conductivity measurement procedure mentioned as: " Temperature-compensated measurement may be performed after suitable validation".
What is validation in this case and how to perform the validation?

A1: Don't focus on that one sentence. The European Pham text is unchanged for many years. The full text is...

"Measure the conductivity without temperature compensation, recording simultaneously the temperature. Temperature compensated measurement may be performed after suitable validation."

"The water to be examined meets the requirements if the measured conductivity at the recorded temperature is not greater than the value in Table 01269 (or 0008).

There is no change to the procedure. You measure the temperature and conductivity (without temperature compensation).

Compare this measured conductivity to the limit from the Table (from the PW or WFI monograph).

The statement "Temperature-compensated measurement may be performed after suitable validation" is not a requirement. The test is to use no temp compensation.

How to validate a temp comp measurement" is challenging. Temp comp measurement is based on 1) temperature, 2) non-temp compensated conductivity, and 3) the proprietary algorithm developed by the conductivity manufacturer - and no 2 manufacturers have the same algorithm.

I advise to use non-temp compensation for conductivity to demonstrate compliance with the finished waters, i.e., monographs.

I advise to use temp compensation for conductivity for all waters prior to final release, for process control. This is written into recently harmonized chapters USP 644 (not 645), EP 2.2.38 (general conductivity chapter), and JP.

EM Time Frame

When it comes to Environmental Monitoring is there any standard that mentions how far apart sampling should be? For instance, let's say that you are in an ISO 7 clean room and your procedure tells you to monitor the area twice a month. Is there something that says you need to make sure there is a week, or more gap between samplings or you can't do it on back-to-back days or is this just something that is up to your own findings. I could see why having a week or more gap would be good information because the room has changed more in that time introducing more things, however in a facility that doesn't produce the same things every day and has different things going in and out daily the day to day could introduce change just as well and the information could be just as useful. Any thoughts would be great.

A1: I'm assuming you're producing non-sterile products based on the framing of your question. If you're just meeting the letter of the recommendation, then no, there is no set timeframe. If you're manufacturing multiple products on multiple days over the course of a month, you should probably do a risk assessment based on the products you're manufacturing as well as your patient populations. EM is just a snapshot in time showing the health of your facility so looking at back to back samples with 29 days between doesn't really give you a good picture of the health of that cleanroom during those 29 days.

A2: In addition to the guidance that [name redacted] had provided above, there were several industrial environmental microbial monitoring surveys for nonsterile products that had been conducted by PDA and PhRMA which could provide additional information that might be helpful to your question concerning testing frequencies from a historical perspective. I believe that PhRMA conducted its survey in 1994 and PDA conducted their’s in 2006. However, I do not have copies of these surveys, but I have seen each of these surveys be referenced in a presentation by Dr. Leonard Mestrandrea that is on the PDA website. I believe that the 1994 survey broke out the frequency for microbial testing of liquid orals, solids (e.g.; tablets) and topical products for the sampling of air, compressed air, contact surfaces, floors/walls and purified water. I have not seen any other type of environmental microbial monitoring surveys for non-sterile products since that time.

It would be nice if this environmental microbial monitoring survey was conducted today again for non-sterile products to see if there had been any changes in how environmental microbial monitoring is being

conducted today in comparison to the 1990's and 2000's. I expect some things to be the same, but other things being conducted today may be different from what had been conducted in past surveys.

Sterility test isolators and room

Having been out of the sterile manufacturing/testing arena for a few years I wanted to gauge peoples current opinion on a query that I have as well as any regulatory requirement that may be available and technical position. A similar query has been asked of this group before (Mar 2017) so my apologies for a certain amount of repetition.
Currently I can find no real reference or requirement for a sterility test isolator to be in a controlled/ classified area i.e. grade D or C other than a reference within PIC/S referring to controlled access for staff but not from an environmental perspective - is this still the case? Does anyone know of any other legislation that require this sort of area to be classified (this is solely referring to a lab based isolator for sterility testing purposes)?
I'm aware of some businesses housing isolators in classified areas for sterility testing and was wondering what sort of technical justification or rationale supports this approach given the current position of it not being a requirement? I personally do not understand what the technical rationale could be for this given that isolator usage and control should be appropriately validated to mitigate a requirement for additional monitoring in what should be just a laboratory area.
And finally does this approach not then set precedents which could ultimately force, through various routes, this to be a regulated and auditable requirement?
I would be very interested to hear peoples views and opinions on this topic.

A1: Please refer to the Barrier Technologies section in the revised draft Annex 1 (Feb. 2020) for specific details on the background environment for both open and closed Isolators.

The Annex doesn't differentiate between Sterility test Isolators or Isolators used in manufacturing. I've typically seen Sterility test Isolators in a Grade D background.

4.21 For RABS used for aseptic processing, the background environment should meet at least Grade B. The background environment for open isolators should meet Grade C or D, based on a risk assessment. Airflow studies should be performed to demonstrate the absence of air ingress during interventions, such as door openings.

4.22 The background environment of a closed isolator should correspond to a minimum of Grade D. The disinfection/decontamination programme should be included as a key consideration when performing the risk assessment for the CCS of an isolator. Where additional process risks are identified, a higher grade of background should be considered. The decision as to the supporting background environment should be documented in the CCS.

A2: I have not seen any requirements for this either. Although the expectation that sterility testing be conducted in at least the same environment as how the product was manufactured/filled, (e.g., ISO 5), there is no requirement I am aware of that requires a validated isolator for sterility testing be in any classified room. Therefore, an isolator in a standard micro lab should be fine, as long as the proper controls for maintaining the decontaminated state of the isolator (e.g., during transfers) is demonstrated.

A3: You're right, there really is no standard for the conditions of the room housing the sterility testing isolators. Most companies will keep them in a controlled area, some will even keep them in an ISO 7 environment. Isolators aren't foolproof, albeit the best option for sterility testing. Some firms perform monthly EM in the area outside the sterility isolator as well. USP 1208 is a good reference for this topic.

USP 1208 - Validation of a Sterility Isolator

"Selection of a Location for the Isolator

Isolators for sterility testing need not be installed in a classified clean room, but it is important to place the isolator in an area that provides limited access to nonessential staff. The appropriate location provides adequate space around the isolator for moving transfer isolators, staging of materials, and general maintenance. No environmental monitoring of the surrounding room is required.

Temperature and humidity control in the room is important to operator safety and comfort and is critical for the effective utilization of certain decontamination technologies. Uniform temperature conditions in the room are desirable when temperature-sensitive decontamination methods are employed. Care should be taken in locating the isolator so that cold spots are avoided that might result in excessive condensation when condensing vapors are used for decontamination."

A4: I have worked at sites with sterility isolators in a non-classified laboratory. This was not challenged by agencies (FDA/HPRA). The VHP cycle/load pattern was qualified and routinely re-qualified separately from the production isolators, and glove integrity testing/environmental monitoring was performed for the sterility isolator. In short: it was treated exactly like a production isolator except its location.

Because a non-classified background for a sterility isolator has no impact on product quality, I think this is easily defendable (not so much with production isolators).

A5: USP <1208> STERILITY TESTING—VALIDATION OF ISOLATOR SYSTEMS states:

Selection of a Location for the Isolator Isolators for sterility testing need not be installed in a classified clean room, but it is important to place the isolator in an area that provides limited access to nonessential staff. The appropriate location provides adequate space around the isolator for moving transfer isolators, staging of materials, and general maintenance. No environmental monitoring of the surrounding room is required. Temperature and humidity control in the room is important to operator safety and comfort and is critical for the effective utilization of certain decontamination technologies. Uniform temperature conditions in the room are desirable when temperature-sensitive decontamination methods are employed. Care should be taken in locating the isolator so that cold spots are avoided that might result in excessive condensation when condensing vapors are used for decontamination.

A6: I believe this is a requirement discussed in the revised draft of EU-GMP Annex 1, which is currently under review/comment process. If your product is supposed to be compliant with European regulatory requirements, you may want to consider qualifying the sterility isolator in a classified/controlled room. Although, as mentioned in the previous emails, if the isolator unit is not in a classified room as the risk to product is minimal it can easily be defended.

A7: Majority of the sterility isolators are located in controlled but unclassified areas. VHP is a robust process when validated properly but is a 'surface' process; thus, it is not effective on unexposed surfaces. In particular, you should pay close attention to the media and rinse fluid bottles that are packaged in cardboard boxes and the potential risk of a spore contamination resulting in a false positive. It is a best practice to use double packaged or perform a wipe down of the bottoms with a sporicide prior to VHP.

False positives should be zero in a sterility testing isolator and if this is not the case, then any agency may request that your isolator be in a classified area.

recovery factor

In recent audit at our oral solid dosage facility, we received one observation regarding recovery factor.

We are taking swabs of production equipments during cleaning validation studies and do bio-burden test. We have done recovery studies by spiking coupons with different stains of m.o. The acceptance criteria for such recovery study was >70% and the recovery obtained were near about 90%.

Here the auditor observation is since the recovery is <100%, we should apply recovery or correction factor in the calculations.
Please let me know, how many peoples are applying such recovery or correction factors in routine.
Any reference or guidance on the same will be helpful.

A1: No regulation paper indicates correction factor.
Practice indicates the following:
Below 50% reject the method or swab twice.
Between 50-70% employ a factor of 2
Above 70% no correction needed

A2: As a long-time auditor that must be a generalist to do that job it is always a temptation to step way outside my area of expertise.

Correction factor would work if you had a separate correction factor for each and every organism. Yes, a lame attempt to apply chemistry to microbiology.

Bioburden and filter integrity test

I am new to the industry and I have two subject to conduct.
First is bioburden of pharmaceutical paraffin. Could you give me any guideline how I can start that process. What do I have to consider during the bioburden.
The second subject is filter integrity test. I do not have equipment to do it at my premises. My client wants to carry out the test after production.
Is it even possible to do that?
Do I have to flush the membrane to remove paraffin to provide clear filter?
Will the solvent remove all paraffin from the membrane?
Will the result be reliable?

A1: First there is no value in testing paraffin wax for bioburden.

Secondly filter integrity testing is best conducted in the filter housing before and after the sterile.filtration step. Perhaps if you do not have the capability of conducting a filter integrity test your company is not equipped to make sterile products.

A2: In general, paraffin wax is not susceptible to microbial contamination. It is made mostly from petroleum and is not soluble in water. To use it in a product, it needs to be melted at a high temperature or be solubilized in a solvent such ether or benzene that are hostile to the survival of organisms. Paraffin wax is an ingredient in which no microbial testing is required.

A3: I disagree. Ps. aeruginosa grows in paraffin.

A4: Is it possible to get a water activity assay performed on the raw and final forms of the paraffin (if different)? You could perform a risk assessment to determine whether bioburden testing is necessary in this case.

DI water endo spikes

We have a DI water system and we are seeing endotoxin values throughout the system around 0.250 EU/mL, but little to no bioburden (<1 CFU/mL). I would assume if there are endo spikes we'd see higher bioburden values as well, but that doesn't seem to be the case. Any idea why this would be? I do not have a great understanding of biofilms, but maybe this is what is occurring.

A1: The discrepancy between endotoxin and micro depends on a lot of things, including, but not limited to, the micro sampling method (sample volume, after sanitization of a sampling port or use point, etc.),

location of samples (which may miss biofilms) ; the micro method for recovery (is it recovering stressed organisms in the system), and where and how the endo tests are being performed. I have a number of current clients that have seen similar situations and it was determined that the micro portion of the testing was not adequate to detect low levels of organisms or biofilm. So, if you can provide additional info someone in the group may be able to assist.

Discrepant MLT replicate results

One of my consulting client's customers obtained some unusual replicate results from USP <61> testing of my client's products. Their customer recovered <10 cfu/gm for one replicate and TNTC cfu/gm for the other replicate. My client's customer deemed this to be OOS, and repeated the testing. The repeat test had replicate results of TNTC cfu/gm and TNTC cfu/gm. My client's customer has also performed randomized sampling of multiple drums of the same product, with most showing very low recoveries from the majority of the drums, but a couple showing TNTC (I don't know if the MLT replicates were both TNTC). My client has sold the same lot of product to multiple other customers who have reported no issues.

My position is that the original "<10 vs TNTC" is an invalid test, and in conjunction with the other odd recoveries from the same lot, indicates a testing issue. It's worth noting that my client's customer plates an entire 1 mL of the 1:10 diluted product onto the surface of a plate. In my opinion, this is highly unusual (it's usual to do a pour plate of 1 mL or a spread plate of 0.1 mL).

I'd appreciate any thoughts our QC Microbiology colleagues might have.

A1: This sounds like a pipetting error by the analyst for the sample results of <10 cfu/gm if the other recoveries were TNTC. What were the negative control results? Also, if I understand correctly, the lab is performing a spread plate of 1.0 mL? The surface would be flooded and once inverted for incubation, do they not see leakage of the plate?

A2: Could you share what the organism id was (pure or mixed)and what the material is as this better indicate source if the contamination?

A3: Instinctively I’m in agreement. I’d be inclined to look at the historical data to support the position. But yes, if dilutions are being performed incorrectly (or not at all..), that would explain results that are varied to that degree, in the absence of any root cause that has been investigated.

A4: Need more info [name redacted]. Such as how drums are sampled, whether the drum contents are hygroscopic which could cause non-homogeneous contamination, the results of negative controls, historical data, etc. I cannot simply point to a lab issue with the limited info provided.

A5: I know spreading spore-forming bacteria can cause this type of result if you truly had a lawn/TNTC of one colony morphology. Some of these critters, like Paenibacillus spp. can take over an agar plate quickly from just one-two cells.

A6: As part of their investigative sampling, they sampled square root plus 1 drums. I don't have specifics regarding the total number of drums my client shipped to the customer, but I recall that it was a fairly low number (maybe a dozen). The product is oil-based, so hygrophobic. We were not informed of any negative control issues, so I would have to assume that these came out okay. As far as historical data, for this particular product, my client has shipped multiple drums to several other customers. None of these customers have reported any issues. The recovered organism was a pure culture of a bacterial spore-former.

A7: The material is oil-based and hygrophobic. The contamination was a pure culture of a bacterial spore-former.

A8: In reading the responses that you have received, there are definite issues that are occurring.

For one, I do have questions as to why a 1.0-ml aliquot was used to do a spread plate plate count in which the agar plate was 100 x 15 mm. For this size of Petri dish, I would recommend the usage of a 0.1-ml aliquot for a spread plate for a 1:10 dilution. If the Petri dish was 150 x 15 mm, I would be open to use a 1.0-ml spread plate aliquot. Does your client have microbial count validation test data showing that a 1.0-ml aliquot of the 1:10 dilution of this raw ingredient did not have an adverse effect on the spread plate counts in agar plates that have a plate count size of 100 x 15 mm? I'm assuming that they have this data showing no adverse effects.

If you are compositing the square root plus one into a single sample, it is very possible that there was a compositing or plating issue. However, it is also possible that the contamination is not homogeneously distributed throughout the batch. By composting all of the samples into one sample container, you may have diluted the contamination because it looks like that some sample containers may contain a low level or did not contain the contaminants while others had a high count. The only way to tell is to conduct a microbial count on each of the square root plus one sample container to verify that the contamination was not homogeneously distributed in the square root plus one containers. I have seen this to occur in many raw ingredients in which the contamination had not been homogeneously distributed in a production batch, Because the customers of the client had not detected the presence of TNTC levels, they may have been receiving a portion of the batch that is either free of the contamination or has low levels. As far as the historical test data, it is possible that this material has a very good microbial test history. However, it does not rule out that an issue had occurred in this production batch resulting in having a portion of the batch to have TNTC levels because of dirty shipping containers.

It is not unusual to detect the presence of Gram-positive bacilli in anhydrous oil raw materials. They may not proliferate in the oil, but can survive.

A9: Wonder at the dilution dynamics of this hydrophobic material. Does the protocol obtain a well dispersed stable suspension?

A10: Dealt with a similar situation as a Pharma lab -
For lab - besides the method -. If ok, not sure lab result can be negated if negatives are ok and all repeat testing ok too.
Sampling here is important to examine- location, sample containers (sterile ones or not) sample equipment (single use etc. I've seen where non sterile raw materials are quite exposed during sampling .... result may be true but need to rule out it didn't happen on site.

The container was culled, the remainder of lot tested and within limits was released- RA of the impact on the final product / theoretical cal of what impact might be on end product (non sterile/ patient pop etc) for internal Q .
The material was on increased level of testing for the next few batches.
Your client might expect increased scrutiny from their customer / complaint - and depending on impact, they may request cherry picking of lots with the low levels . As for other customers not getting the same results not all industries that use a raw material have the same incoming checks - Pharma vs cosmetics and also nature of the contaminant in their product.

A11: It's a Lysinibacillus species. Definitely not a spreader.

A12: This is a matter of detailed investigation that can cover severals points.
1. Sampling bag/tools-sterile or non sterile.
2. Pipettes/plates-properly sterilized.
3. Pooled sample or sample was taken from a single or two drum. (Pooled sample is recommended)
4. Negative control- should show no signs of growth.
5. in case any of the customer has not reported any issue. Then this might be a testing error due to pipette contamination. As it seems lab is reporting TNTC. Lab is not making serial dilutions of the sample.
6. If it was sample from 1-2 drum, may be that particular drum was contaminated. Which gets mixed to a non contaminated drum. And finally the counts gets reduced. Hence customer didn't get high counts.

7. Oil based samples are different treated, may be due to addition of a neutralizer has contaminated the product at the time of testing.
8. Company personnel has to look critically at each aspect. an expert microbiology insight is required to complete follow up of each experiment and investigation.

A13: It is my understanding that the members of the genus called Lysinibacillus used to be classified as members of the Bacillus genus. Their normal habitat seems to be soil. It is possible that some of the product containers could have become contaminated with dust that contained the Lysinibacillus isolate. Without knowing the source of your oil, it is possible that soil is also the source if the oil had been obtained from a botanical plant.

The most common isolate of this genus seems to be Lysinibacillus sphaericus which used to be classified as Bacillus sphaericus. From my experience, this organism is not a spreader.

A14: In addition to all the good points previously noted, a few things need clarification / standout

1: Tech Error -
In referring to replicates, I presume that by this was meant the repeat aliquots of the exact same material preparation at whatever dilution was appropriate. Assuming a standardised suitable test system is in place such a discrepant result is likely to be a technical / lab error as irrespective of the presence of non-homogeneous microbial distribution in original bulk material, a suitable test system / preparation technique, should allow for reproducible counts in replicates.
Common Technical Sources include ;
Suspension Issues
Poor re-suspension technique in the test system - unsuitable diluent and/ or poor or non reproducible mechanical mixing, inadvertently letting dilutions settle between replicate tests etc.,
Contaminated Reagents / Equipment
Contaminated Pipette or Pipette Tip,
Spread Plate Issue - A contaminated hockey stick etc, or an agar plate surface contaminant (unnoticed single colony etc) already on the plate surface that was inadvertently lawned / spread during the spread plate technique. Less likely but possible would be outgrowth of a contaminant before the plate surface had time to dry but such effects are normally localised to parts of the plate unless it is a spreader and apparently it's not.

2: Failure Investigation Outcome
For repeat / investigational testing you mentioned that some high counts were encountered - I presume replicate test were conducted during the approved Phase II investigational program. Were the results of these reproducible i.e. no discrepancy between replicate tests? If that was the case it would support suitability of the test system and would focus the investigation on the test conditions relevant to the specific MDD result.
I'm also assuming that your client has no historical evidence that such discrepancies occur in the past that might implicate the suitability of the test system used for the particular material.

3: Microbiological Profile
What type of flora are normally associated with this product - i.e. when growth was recovered, be it low or high level by your client in the past and also by their customer during the recent failure investigation, were mixed or pure populations encountered (morphotyping or gram staining would be sufficient to provide an indicator). Is it normal to have very few types or mixed flora?

4: Pure Culture Lysinibacillus spp
I am struck by the fact that the original contaminant event involved a "pure culture" unless the material provides a highly selective advantage for Lysinibacillus spp., Comparative growth challenges using recovered isolates or historic data from 3 would quickly confirm this. Were isolates recovered during retesting only of Lysinibacillus spp or mixed sporeformers or others. Personally, I would be surprised not to see some mixed growth during high level contamination unless it was a localised point contamination e.g. in a material drum etc or an upstream specific process issue that allowed outgrowth of "one

contaminant" ,either way that would still not explain the replicate discrepancy. Based on available info I would think it's likely to be a point contamination of some test reagent / test item, such a pipette tip, hockey stick or colony on an agar plate as mentioned in 1 above .

Anyway, hope this together with all the other input helps, as always with Micro there are lots of maybe's or what if scenarios, - that's what makes it such a great subject.

Scaling back micro testing / risk assessment

I am seeking a guidance (if one needs to be purchased, that's fine) regarding performing risk assessments to justify removing micro testing and / or justifying loosening specification limits. The product has very strong antimicrobial properties and is actually a disinfectant. I'm typically working with aseptically manufactured product, so this type of question / justification is somewhat new to me. I saw a year of data that was completely clean thus far. Not sure what constitutes "enough" data here to make this justification. Other considerations such as water activity would be addressed in the risk assessment. If there are other considerations for disinfectants, I would greatly appreciate input / viewpoints.

A1: "Disinfectant" per se does not offer an assurance of no biological contamination. Think your 1st considerations would be why - what are compelling needs to eliminate QC at your end? Why loosen a spec - why the current spec? Assuming your testing has value, you're proposing to delegate your quality responsibility to your supplier.With justification - think you'd need to understand, with supplier, their risk assessment of disinfectant production, delivery and use. What are their validated control measures, testing is performed and change control. how do they know/assure the quality of what you get?

Testing of salmonella in non-sterile pharmaceutical product

Want to know significance of using 10 gram sample in test for Salmonella species instead of 1 gram for others and why using 10 ml rappaport vassilidis broth and 0.1 ml inoculum instead of 100 ml and 1 ml, respectively?

A1: The larger sample size is Salmonella is a serious foodborne pathogen cause infection with <100 CFU and the greater sample size increases the sensitivity of the test.

Yes, you can use 1 mL in 100 ml.

Raw material testing question USP<60>

Do we need to perform USP<60> B.cepacia testing for raw materials used in preparation of oral liquid finished products? As per USP<60>, The tests are designed to determine whether a substance or preparation complies with an established specification for microbiological quality and/or to evaluate whether products—especially those for inhalation use or aqueous preparations for oral, oromucosal, cutaneous, or nasal use—contain members of the Bcc.

I guess preparation does include raw material, but just want to make sure if i am interpreting this correctly.

A1: If you believe that because of the origin and physicochemical attributes of the pharmaceutical ingredient, it has a high potential to add Bcc to the product, yes run the test or modify the compendial testing to identify any objectionable microorganisms.

Other potential Bcc sources are the purified water and inadequate process equipment cleaning and storage.

A2: Have you performed any studies showing you can recover Bcc from the finished product or raw material? Is there anything in the manufacturing process that would mask the growth/presence of Bcc

either in the raw materials or in the finished goods? I'm inclined to say you should be testing your raw materials and your finished good and if you don't see any recovery of Bcc for a year (pick a timepoint) through a risk assessment (anti-microbial, low Aw, etc) you may be able to say the Bcc contamination wouldn't most likely come from that raw material. If the raw material is aqueous based, I would say you should definitely test both.

A3: I guess performing a suitability study will help prove the recovery of BCC in raw material.

Since it is dry raw material, the chances of BCC contamination are fairly low. And if the testing is done on the finished product, then it takes into consideration all the process steps and risk points.

Sterility test

I use the steritest canister sterility testing system for a product. After testing about 5 of 20 vials the filtration becomes slower and more difficult. The diluent used is fluid A. The product is viscous. Can I divide the test and use 4 steritest units each filtering 5 vials? Do I need to revalidate after this change to the test?

A1: What color canister are you using? We have blue for easy to filter products, red for anti-microbials and green for viscous products. We also have sterile IPM that can be used as a solvent. Other solutions are to slightly warm the product or dilute it into more Fluid A or D. From your description, the product could be binding to the membrane or it could just be too viscous with a dilution.

A2: Yes, you can divide the sample. You can test as little as 1 vial per canister set, but it is more cumbersome and risky due to all of the handling. If you validated more vials, but use less using the same method (filter type, same rinse strategy, etc.) you do not need to revalidate. If you change the method you will need to revalidate.

BCC is a part of the specification

We have product like Azithromycin oral suspension, its dry powder form.At the end user product is going to be in dry powder form, patients add 'X' amount of water as per label.

My question is, should it consider aqueous preparation or non aqueous preparation & what about its TAMC,TYMC & Pathogen limit.it is necessary to perform BCC pathogen test is part of the specification.

USP 60

We have a sterile manufacturing plant for injectables. All of our products are being manufactured sterile.

Do we need to perform USP 60 testing on raw/ bulk material that is being used for the production of injectable products? Also, What about the finished product?

A1: You may want to include microbial enumeration testing on certain raw materials based upon a microbiological risk assessment but there is no need to test specifically for Bcc since it will be removed/ neutralized during your validated sterilization process. Your finished product will then be subject to a USP chapter <71>/EP chapter 2.6.1 style sterility test, not a specified organism test for Bcc as per USP chapter <60>.

A2: The answer is no!

A3: No, USP 60 is meant for non-sterile, aqueous solutions only. Tony Cundell from the USP committee has confirmed this on the PMFList.

A4: Generally, USP chapter <60> Microbiological Examination of Non-Sterile Products Tests for Burkholderia Cepacia Complex became official as a means for testing drug components and/or final preparations for the presence of Bcc. USP chapter <60> builds upon the test methods and acceptance criteria recommended in USP chapters <61>, <62>, and <1111> to provide a relatively simple and reliable test for the detection of Bcc associated with pharmaceutical manufacturing processes.

If it's a Sterile Raw material tailgate sample /Sample collected during usage and annual 3 lots testing is in general practice.

If it's a non sterile material please follow √n+1 sampling and testing.

Suggest to perform a formal risk assessment with FMEA tool, based on Vendor Audit, Vendor agreements, respective samples number of rejections, CAPA compliance and effectiveness. Based on the previous trend and performance of the material/vendor, evaluate the risk and sampling /Testing frequencies.

A4: USP chapter <60> is for non sterile product but as per my knowledge, you should also consider "Monograph" of individuals raw materials And USP chapter <71> for sterile FP products.

If monograph of raw materials requires microbial contamination test, it may possible to avoid that test?

Environmental monitoring, action limit exceeded, risk assessment

When dealing with deviations occurring during microbiological environmental monitoring in facilities manufacturing non-sterile pharmaceutical products, how can we do calculation of the number of microorganisms from the deviating sampling location that would theoretically be present in the product, or product bioburden testing results.

A1: You cannot make a theoretical calculation/derivation. If you recover the a microorganism in an EM sample and also recover the IDENTICAL microorganism then you can infer a probable cause and effect.

This also assumes that you are able to determine the the location of the EM sample excursion had a direct influence or contact with product. This would be part of your investigation. Then you also have a multitude of other situations (lack of recovery in EM or bioburden, external influences, multiple recoveries, etc). Keep in mind very few micro investigations lead to the exact root cause. This is especially true in non-sterile manufacturing applications.

EM and bioburden (and microbiology, in general) is funny like that (not always “ha ha” either).

Sadly, no formula or theoretical calculation can be applied.

A2: Environmental monitoring data cannot be directly correlated to product contamination. Your investigation should take into consideration the type of sample (ex: air vs surface) sample location, type of organism, and most probable root cause of the excursion. Is your sample location close to product stream? Is the organism mold? Gram positive? Gram negative? Is the root cause related to an activity that would impact your product quality/patient safety? You may wish to consider increased product testing as well.

If you wish to provide more information, the group may be able to help further.

A3: My calculation of the number of bacteria in a controlled environment like a non-sterile product manufacturing facility entering the product would be close to zero. Yes that got your attention!

I would refer you to USP <1115> Bioburden control in Non-sterile Pharmaceutical Product for a discussion of the hierarchy of microbial contamination risk.The pharmaceutical ingredients, water system, and process equipment cleaning and storage are much greater sources of contamination.

Solid oral dosage- Hold time study

We are performing analysis for hold time samples (Coating solution, Granuation media, Binder solution and wet mass, etc.,..) as per finished product analysis means TAMC, TYMC and pathogen analysis. My question is, can we test only TAMC and TYMC for these hold time analysis (stations initial 6th hour, 12th hour 24th hour and 48th hour.)

A1: I think yes, perform test for pathogen for all sample.

A2: In my experience only TAMC is sufficient.

This is because the process equipment and water are the primary source of the contaminants and as the materials have a high water activity, bacteria will outgrow fungi.

FW: [PMFLIST] USP <60> Question

I was wondering if anyone knows if there are plans for USP <60> to include further information/clarification regarding the growth promotion in the Interpretation section? Currently, the possible presence of Bcc is indicated by the growth of greenish-brown colonies with yellow halos or white colonies surrounded by a pink-red zone on BCSA. We are finding the B.cepacia organism does have the greenish-brown colonies with yellow halos on BCSA, but we would not call the B. multivorans or the B. cenocepacia colonies white. We are getting a pink/red zone, but not with white colonies. Is anyone having a similar experience?

A1: The difference in the color of colonies on BCSA might be due the difference between B. cepacia complex wild type isolates that are normally found in a manufacturing environmental and B. cepacia complex isolates in human clinical samples. You have to remember that BCSA was originally developed to isolate Burkholderia cepacia complex isolates in clinical samples from cystic fibrous patients. I would not be surprised that you are seeing a difference in colony color due to the origin of the B. cepacia complex isolate. It could be due to the fact that B cepacia isolates in a manufacturing environment could be stressed resulting in a difference in colony appearance on BCSA in comparison to clinical isolates.

From practical experience, I have found not to use the presumptive biochemical reactions on a selective/ differential agar for determining a presumptive identification of an isolate. It can be used as a guide for identification, but I still would conduct a gram stain and a phenotypic/genotypic identification to determine a final identification on any recovered isolate that is able to grow on a selective/differential agar no matter if it is or not showing typical biochemical reactions.

A2: We are actually using the strains required in the USP for Growth promotion testing , but we are not getting the expected color changes on our BCSA. B. cepacia looks as it should, but the other required organisms B. multivorans and B. cenocepacia take on a different appearance.

We do plan to perform further ID on any growth recovered on BCSA during routine product tetsing, but we also perform QC testing on all lots of the media for release. We are concerned that our recovery of these two organisms is not matching the interpretation for the possible presence of these organisms on BCSA as listed in the USP.

A3: I have seen that most microbial media manufacturers of this agar are only using Burkholderia cepacia ATCC 25416 as a growth promotion organism for BCSA. However, I have found that there is a BCSA microbial media supplier that is using Burkholderia multivorans and Burkholderia cenocepacia as part of their growth promotion organisms in addition to Burkholderia cepacia for this agar. They state that Burkholderia cenocepacia ATCC® BAA-245 colonies on this agar will appear as small pink colonies with gray-purple centers; slight yellow halo reverts to pink halo. Burkholderia multivorans ATCC® BAA-247 will appear on this agar as small pink colonies with gray-purple centers; slight yellow halo reverts to pink halo. This BCSA microbial media supplier does not make any statement on their technical specification sheet

indicating that Burkholderia cepacia complex members (B. cepacia ATCC 25416, B. multivorans ATCC BAA-247 and B. cenocepacia ATCC BAA -245) will appear as white colonies surrounded by a pink-red zone. It may be possible that other members of the Burkholderia cepacia complex will appear as white colonies surrounded by a pink-red zone and not the three strains indicated for growth promotion usage for BCSA..

Have you ever considered the possibility that USP Chapter 60 could be incorrect concerning the appearance of Burkholderia cepacia complex members especially Burkholderia multivorans and Burkholderia cenocepacia on BCSA? I know that I am talking hearsay in stating that the USP could be incorrect concerning the color reactions of these strains on this particular selective/differential agar.

I would recommend that you do not worry about the BCSA color reactions for B. multivorans and B. cenocepacia as long as you are able to demonstrate growth of these strains on this agar.

Pseudomonas maltophilia / Pseudomonas beteli

Wanted to present a question regarding the appropriate handling of an identification. A purified water system was tested with MALDI-TOF. The official ID was Stenotrophomonas maltophilia. Spec indicates absence of Pseudomonas spp. Stenotrophomonas Maltophilia is a heterotypic synonym of Pseudomonas geniculata.

Should this be investigated? Would this result cause the test to fail per the specification?

A1: My apologies for the error in the subject. I've corrected it here.

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Should this be investigated? Would this result cause the test to fail per the specification?

A2: Taxonomy has tripped up the ID world many times in the past 20 years as systems don't always keep up as quickly as the taxonomic world. It's gotten much better with all the advances in the digital age. We know S. maltophilia WAS Pseudomonas and Xanthomonas at one time. With more and more definition thanks to sequencing, these organisms get moved around when they don't fit the "current" taxonomy. In my view, S. maltophilia is the current taxonomy, therefore the current identification. Is it closely related to Pseudomonas, Iof course, but for ID purposes I'd call it S. maltophilia.

Here's a link to an explanation: https://en.wikipedia.org/wiki/Synonym_(taxonomy)

" Unlike synonyms in other contexts, in taxonomy a synonym is not interchangeable with the name of which it is a synonym”

A3: If you have a spec that states absence of this organism, then you failed the spec and it must be investigated.

A4: At one time, Stentrophomonas maltophilia was known as Pseudomonas maltophilia. As far as Stenotrophomonas maltophilia being a heterotypic synonym of Pseudomonas geniculata, I'm unaware of it.

However, it is my understanding from the scientific literature that Pseudomonas geniculata had been transferred to the genus Stenotrophomonas is now considered to be a synonym of S. maltophilia (Van den Mooter and Swings, 1990; Anzai et al., 2000).

Stentrophomonas maltophilia is a common Gram-negative bacilli in circulating ambient purified water systems. From my perspective, the presence of any Gram-negative bacterial species in a purified water system should be investigated. I would recommend that your water test specification be updated for the absence of all Gram-negative bacilli because there are other common Gram-negative bacilli species that can be isolated from ambient purified water systems that are not members of the Pseudomonas genus.

A5: I'm in complete agreement with investigating every gram negative recovered from the PW system. I mentioned to the lab that I'd like to move towards that sooner rather than later.

[name redacted], I'm a bit confused - if spec states absence of P. aeruginosa and B. cepacia, only / specifically, and the ID was S. maltophilia, are you saying this would fail that spec?

A6: This appears to be an extreme view. If you find bacteria in a PW system they will most likely be Gram-negative, oxidase- positive bacterium. Remember that the microbial count may be as high as 100 CFU/mL so the PW is not sterile.

The term investigation has compliance implications. Do you really mean that the company should open a formal investigation and not release any products implicated in the finding of a Gram-negative bacterium in the PW System?.

A7: I would agree that it does seem a bit extreme to hold up product because a gram negative was recovered in the PW system. And your point with it being in an official GMP "investigation", and those implications, is taken. In terms of controlling biofilm and responding to organisms that typically contribute to the formation of biofilms - without going overboard with the approach - I'm hesitant in doing nothing. Will need to find a middle ground. I'm just looking at a history of flora in PW .... would be surprised if biofilm is not already an issue. High diversity and population is suggesting a pretty established flora.

I never appreciated how clean aseptic facilities were until now.

Criteria for Drain monitoring in non sterile dosage forms

I am working in a frim which predominately manufacturers non sterile dosage forms. I know that drains in grade C and D areas are to be tested and proven to have free of any pathogens. These are as per the applicable guidelines of either PDA/ USFDA/ or EPMEA for sterile formulations.

My questions is what should be limit and testing criteria used for proving the drain are free of any pathogens and it's frequency for a non sterile formulations company. Also pls. Share the reference guidance if any either in document or link format for me to evaluate the same. As per my knowledge, drains have to be monitored for TAMC and TYMC parameters with a internal limit of 500 CFU / Ml.

A1: Drain point monitoring recommended by Schedule M only. No other guideline recommended by drain point monitoring and monitoring requirement only free from pathogens.

A2: There is no any guidelines stating monitoring of Drain is mandatory for non sterile facility, however you can still incorporate cleaning/ disinfection of drain points with possible frequency (e.g. once in a week) by your regular disinfectant for control measure and to minimise the risk.

A3: Please note that some jurisdictions do not permit placing disinfectants into the drain and you may be fined and you may spend time in jail for such actions. There are many things that may not be permitted into the sewer system, as these substances may deplete or decrease the sewer flora. Please verify with your local authorities prior to placing disinfectants, chemicals, product residues, and restroom cleaners into the sewer. It may be easier to drum the waste and close the sewer lines.

Validating IPA use in sanitization of gloves during microbiological testing

Has anyone been asked to show data for the effectiveness of IPA70% when used on gloves "as needed" or "occasionally" during micro testing. The amount of time that the IPA is in contact with the gloves before they are used is most often 10, maybe 20 seconds. So again, has anyone been asked/required to validate IPS's use under these somewhat inconsistent time frames?

A1: Busy Work!

A2: To establish a time-period for usage of an alcohol hand sanitizer, most companies will use ASTM E2755-15 Standard Test Method for Determining the Bacteria-Eliminating Effectiveness of Healthcare Personnel Hand Rub Formulations Using Hands of Adults against either Serratia marcescens or Staphylococcus aureus in which there is greater than a 3-log reduction in a 30-second exposure period. After this had been established, companies will then use ASTM 2316-16 Standard Guide for Assessment of Antimicrobial Activity Using a Time-Kill Procedure which is a suspension test is conducted against various types of organisms to verify that the product is indeed active in the desired time-period by having either a 99.9 or 99.99% reduction.

Based upon this conducted testing, the manufacturer of the alcohol hand sanitizer will recommend a time period of usage on the label for their product. From my perspective, most pharmaceutical Quality Control microbiology laboratories are generally not equipped in how to conduct ASTM E2755. I do not see the point as to why a pharmaceutical microbiology laboratory would be requested to conduct this testing again if you are following the product usage directions of the manufacturer. I agree with Tony' comment that it is busy work and a waste of time. If you are deviating from the time period of product usage exposure for an alcohol hand sanitizer, I would think that you have bigger issues going by using an alcohol hand sanitizer in less time than what had been recommended by the hand sanitizer manufacturer.

A3: To validate IPA use in sanitisation of glove and contact time, required a study!.

Prepare and execute a protocol with the following parameters which answer your all the questions and also it strengthens your regulatory audits requirements/reference.

1) Determination of contact time: Take 3 sterile test tubes and add 9ml of days disinfectant and 0.1mL/ 1mL of 1000CFU of each test organism in to tubes numbered as 5min, 10min, and 15 min.

After 5min draw 10ml individually and filter through 0.45micron filter, place the filter on individual D/E agar plate and incubate it for 20-25and 30-35 Temperatures respectively.

(3log reduction is acceptable and IPA can kill only vegetative bacteria IPA is not the sporicidal) 2)

2) Determination of Log-reduction for use dilution:

Use the contact time from the above method Prepare10^6 suspension of required test organism as per USP. Add 1mL in 9 mL of disinfectant and allow contact time as determined above Take 1ml of above test solution and add into 9mL of D/E broth. Take 1ml of above test solution and add into 9mL of D/E broth. It gives you 10^4 concentration filter the above entire test solution through 0.45micron filter, wash with 100ml 1%Peptone. place the filter on individual D/E agar plate and incubate it for 20-25and 30-35 Temperatures respectively.

(3log vegetative bacteria and 2 log reduction for yeast and mould is acceptable)

3) Select the required coupon surfaces like SS, Glove, PP, Glass I, PVC and Etc... And perform the recovery study by spreading with 10^7 to 10^6 required organisms dilution, take the swabs and performance the recovery study

(30% Recovery is acceptable)

4) Perform the Hold time study with the days of 0,1,3,5,7,9,11,13,15

Now you have the complete Disinfectant anti microbial efficacy study with required contact time validation report.

Last and foremost thing, Intermediate sanitisation of glove with 70%IPA is to avoid any cross contaminants carry forward and to have confident aseptic operations.

Removing hyphae from A. braziliensis for AET test

Does anyone have advice on the use of steel wool for filtering A. braziliensis \, i.e. how did you set that up. There is also mention of an alternative method for this filtration in <51> , so if anyone has thought on alternative methods that they are using, it would be greatly appreciated.

A1: Nothing to do with filtration - food for thought from an old mycologist who thinks fungi never get enough pressOne alternative method uses hyphae (esp. blended) as inoculum rather than spores (e.g.https://jcm.asm.org/content/21/4/509.short). Certainly not as easy as with a simple spore suspension, theoretically focuses on the immediate event of spoilage - hyphal growth - rather rather than effective surrogate dormant/germinating conidiospore. Literature and experience finds antifungal compounds both more and less effective vs hyphae than spore inoculum, challenging intuitive expectation that spore inoculum is worst case.

A2: I was an original author for ISO 14730, Ophthalmic optics — Contact lens care products — Antimicrobial preservative efficacy testing and guidance on determining discard date. The standard simply states that you can filter the spore suspensions through sterile glass wool, cheesecloth or gauze to remove hyphal fragments. Pack a filter funnel with the material you use for filtration, and pass this your suspension through it. This will catch the hyphae and most of the spores will pass through. Just sterilize what you use first.

UPS1207.2

Could anyone familiar with the above method(Package Integrity Leak Test-Microbial) share helpful/useful infos & experience in order to execute it in a non-sterile manufacturing facility?

A1: What is your requirement? Please raise your query about CCIT. Will try to answer it.

purified water in tank

We have to perform a not ordinary maintenance to our purified water system. The operation is critical, so we have to qualify the system as new installation (IQ, OQ and PQ).
During the PQ - phase one, we can't use the water for the production, and we have not a second purified water system as backup.
We can bring a tank, refilled with purified water, from our associate company in an other location.
We usually test every week their water quality.
Do you suggest to test every day, during PQ-phase one the microbial quality water of the tank, or, because we know the quality of their water system, we can perform a reduction frequency test?

We are an oral solid non sterile manufacturing.

A1: You must test the microbial quality for 30 continuous days. According to your results you can set a routine testing frequency and alert limit.

A2: I would think you need to consider a high frequency sampling strategy for bioburden than just daily samples . If you ship in water, you are effectively employing a batch type system in which you need to provide full traceability between product and quality of the water used in each stage of the process.

The ability of a wide range of oligotrophic bacterial types to proliferate in low nutrient environments such as water (including PF) is the reason that we go to such lengths validating PF & WFI water generation circulation & storage & systems. The same considerations will also apply to the batch of water provided by your sister plant. So, Bioburden Outgrowth will be the principal concern rather than Analytical Parameters (TOC, pH, Conductivity).
You apparently neither have the equipment or time available to conduct a full validation of an alternative system and so by using what is effectively a bulk water supply from an alternative source you must compensate for not having a validated system by doing comprehensive / regular bioburden testing, that reflects the actual quality of the water during holding and particularly at the time of use. The sampling frequency will be reflected by the water usage pattern.
Unless you have engineered special holding conditions (recirculation, ozone, UV, heat etc) on your temporary tank / reservoir system, it is unlikely that you will be able to guarantee the Microbiological quality of the water for prolonged periods (depending on your actual conditions even a day could very well be challenging).
Initial bioburden level & type, hold time, hold temperature and the cleanliness of the receptacle and associated pipework etc will all contribute quality at point & time of use.
While an initial hold time study might help indicate the maximum acceptable hold -time, I would think that the actual water quality will need to be verified for each usage, with little opportunity to reduce the testing regimen as there are just too many independent variables (sister plant water quality, conditions of transfer, holding, equipment cleanliness etc) and too little time to establish reproducible performance. You will have to establish both the initial & usage (Point of Use & Time of Use) data for the quality of such water.
A Frequent replenishment coupled with shortest practical hold-times possible together with regular testing at Time 0 and time(s) of use are indicated here.

A lot of work but necessary.

A3: In addition to the above recommendations.

My suggestion is to monitor seasonal variations is must and important for the Quality of the Product.

It means, first time you should monitored for a year, based on the one year trend data with results outcome (OOL/OOS) you can rationalize your testing frequency.

Based on the consumption and criticality, follow the bracketing approach for sampling and testing.

A4: As per the PQ phase 2 the PW system shall be used for Operation.During PQ water quality should be monitored continuously as per the protocol 2- 4 wks.During this period any ool observed above the phama acceptance criteria shall be recorded and investigated, and repeat the Validation process.When you bring water from other area the quality of the water should be tested on daily purpose if transport validation not done or the water not qualified.

Do i need to perform AET?

The following is taken from US <51>
The concentration of an added antimicrobial preservative can be kept to a minimum if the active ingredients of the formulation possess an intrinsic antimicrobial activity. Antimicrobial effectiveness, whether inherent in the product or produced because of the addition of an antimicrobial preservative, must be demonstrated for all injections packaged in multiple-dose containers or for other products containing antimicrobial preservatives. Antimicrobial effectiveness must be demonstrated for aqueous-based, multiple-dose topical and oral dosage forms and for other dosage forms such as ophthalmic, otic, nasal, irrigation, and dialysis fluids

We have a customer with a new non-sterile multi use aqueous product - Nasal spray. They are saying that it does not have a stated preservative and will not need AET as part of development or stability submission data.

The product will contain Ethanol and EDTA.

1. Should these be termed as preservatives or just inherent antimicrobial atributes from the product formulation and then AET needed or not?

From the paragraph above it says "Antimicrobial effectiveness, whether inherent in the product or produced because of the addition of an antimicrobial preservative, must be demonstrated for all injections packaged in multiple-dose containers or for other products containing antimicrobial preservatives." This is not the case for me.

It then says "Antimicrobial effectiveness must be demonstrated for aqueous-based, multiple-dose topical and oral dosage forms and for other dosage forms such as ophthalmic, otic, nasal, irrigation, and dialysis fluids". It is not clear if this is required for added preservatives or also applies to inherent antimicrobial properties of the product.

1. What is your interpretation on this and if we should be performing AET?

If it is multi use then i thought that preservatives must be added, however if it is inherently antimicrobial and a multi use product then you still need to prove this with AET.

We have not come across a multi use non-sterile product not having a preservative and a customer say that AET is not needed.

A1: There are many instances that so-called self preserved multiple-use, aqueous, non-sterile products have been found to be contaminated with a bacterium or yeast, placed the user at risk, and been subject to recall.

It appears that your customer does not recognize the microbial contamination risk with nasal sprays. I could go as far to suggest that they should not be in this business.

A2: AET is required, whether a multi-dose product contains a “conventional” preservative or not.

A3: The microbiological stability of a multipurpose product must however be demonstrated. The only test that allows to demonstrate stability is the AET.
There are the stability tests in use but they do not have precise indications of how they must be conducted and can only be completed.
In my opinion, the compendial tests of AET lack tables dedicated to multidose products without preservatives. To date, there is no alternative but to use the AET test.
I think it would be interesting to introduce in the pharmacopoeia limits dedicated to products without preservatives that are however valid in adequately preserving the products.
But at the moment they are not provided, so all that remains is to use the limits indicated for products with preservatives.
The nasal sprays can be subject to important pollution in their conservation and must be carefully formulated. So I would use the AET test applying the guarantees of a product with preservatives.

A4: AET is still required for products that don't contain a dedicated preservative. I would advise your customer to also determine which component or components are responsible for preservative effectiveness and the minimum concentrations of those components necessary to convey preservative effectiveness. Self-preserved products often fail microbial enumeration testing during stability due to an overestimation of preservative capabilities of the API or excipients or degradation of those components over the product shelf life.

A5: How have they demonstrated inherent antimicrobial effectiveness to claim compliance with cited language?

A6: One question that i am just following up on is whether Ethanol and EDTA contained within this product are actually defined as preservatives or in this situation would they be classed as having inherent antimicrobial attributes?

If they are clearly identified as preservative ingredients, please provide any reference as i cant find anything, then it is clear cut when i am discussing with the customer.

A7: Ethanol may be considered a preservative but not EDTA, which is a chelator. However, EDTA can potentiate the antimicrobial effects of other preservatives or an antimicrobial formulation.

As for having inherent antimicrobial properties, you may argue the same for ethanol.

I cannot remember your original posts, but if these components are added to a larger formulation, then the final formulation as a whole may or may not impart adequate antimicrobial activity for your customer's needs.

A8: EDTA is not a preservative, but a chelating agent that is an antimicrobial potentiator of preservatives. As a reference for ethanol being a preservative, see Kabara's book called Cosmetic and Drug Preservation. In addition, see David Steiberg's book called Preservatives for Cosmetics. Ethanol is listed in both of these reference books as a preservative.

In general, ethanol at a concentration of 5 to 10% is used as a secondary preservative in most product formulations in combination with other preservatives.

AET for product formulation stored at 4 °C

I have to evaluate the microbiological stability of a formulation that must be stored in its container at 4°C (1 year closed, 1 to 3 months in-use).
Which would be the better method to carry out and evaluate the preservative efficacy test (AET) according to Ph.Eur.?
I would say that carry out this test at 25°C for 28 days is not totally correct.
Have you any suggestion about? How to evaluate it? Should preservatives be used anyway?

A1: I have experience with this from 20 years ago- a topical product that required refrigeration - submitted data on regular challenge test but got push back from FDA asking for challenge test data under refrigeration as well. Their rationale- microorganisms are in dormant or less active state under refrigeration. Preservative activity will slow down when the microbes are not actively growing and also the product will become more viscous- making it difficult to release preservative for activity. So they asked for both data. Inoculate two sets of products- incubate one set at 4 C and second set at 25 C for 28days. Take weekly sample to check for log reduction. To our surprise, we did find slower reduction of mold under refrigeration. Both passed the test since the USP requirement for fungi is no increase. The product was for topical treatment filing in the USA.

A2: The bugs and how they operate in environments is always fascinating. We walk that balance of testing for the sake of testing vs what you describe as an actual, unexpected difference that was illuminated with a study. Micro can be such a gray area...(I guess that's one way to put it!).

A3: The stability study storage temperature is the label instructions, i.e., 4-8 degree C. 20-25 degree for accelerated studies.

The USP <51> Antimicrobial Effectiveness Test is conducted at 20-25 degree C.

Like all chemical reactions preservative effectiveness is reduced at reduced temperature, i.e., each preservative system has a published Q10. Many preserved products would fail the AET if it was conducted at 4-8 degree C. Also the growth of contaminants will be repressed at refrigeration temperature.

A4: You are right in saying that:

1 an AET must be conducted at 25 °C: it is clearly written in the EP and in the harmonized part of the USP

2 considerations: the temperature of 4 °C preserves the vitality of the microorganisms compared to 25 °C. The evaluation criteria between EP and USP are very different (category three for USP, table 5.1.3.-3 for EP). It would still be interesting to see what happens at 4 °C

USP:
Category 3 Products: BACTERIA NLT 1,0 log reduction 14 days - no increase from 14 days at 28 days. FUNGI: no increase at 14 days and 28 days
EP:
Table 5.1.3.-3: BACTERIA NLT 3 log reduction 14 days - no increate 28 days. FUNGI NLT 1 log reduction 14 days - no increase 28 days

3 the preservatives generally have a reduced action at 4°C and also for this reason it would be useful to do an additional experimental test at 4 °C: not as a substitute, but additionally as a check.

A5: Same question, other aspect:
the temperature of 4°C helps to preserve the products from microbiological pollution. In your opinion, can there be justifications for not including preservatives or putting them in reduced quantities? actually I was thinking about this. Or would it be possible to justify a differently stable product because it was kept at 4 °C?
If possible, how can the product be shown to be stable?

A6: What is the in-use instructions? Refrigerate in-between uses? I assume the worse-case would be a 3-month use period. Please advise. This will determine how to conduct the test. Of course, you should have an understanding from the relevant regulatory authority on the expected test regimen and conditions.

I do not understand your question about whether preservatives should be used. Have you formulated the drug product already, or are you in development? In any case, a multi-dose drug product must pass an AET.

A7: Many years ago, I had to run the studies in parallel, room temp storage and refrigeration because the product was stored under those conditions. I also saw slower reduction under refrigeration.

A8: Do not run the test at 4 degree C. If it fails you may create a compliance issue.

A9: No, this is not commonplace, especially when the use period is 1-3 months (from your prior post). If you do not want to use preservatives, why not use single dose rather than multi-dose packaging? Also, I don’t recall you stating whether this is a sterile or non-sterile drug. This may have an impact on your strategy as well.

A10: We are both referring to the same product. LOL.

A11: I was looking for a test strategy that guaranteed me conservation at 4 ° C.
The AET, as correct, should be done at 25 ° C. It is true that refrigeration slows the decay of microorganisms (EP provides for decay much more than USP) and the action of preservatives, so the test would be less in compliance than at 25 ° C, but refrigeration also helps to partially prevent bacterial proliferation .
Also if I keep the product at 25 ° C, I probably also have some non-stability chemical changes.
The comparison with the chemical laboratory will be interesting to understand what happens if the product is kept at 25 ° C for 30 days.

A12: This is a liquid product for oral use (non-sterile) in a multipurpose package.

A13: I have to evaluate the microbiological stability of a formulation that must be stored in its container at 4°C (1 year closed, 1 to 3 months in-use), oral use. I am in development.

Objectionable Organism Swab Location Number - EM

I'm trying to find where guidance comes from when determining the number of swabs required for a specific manufacturing room to test for objectionable organisms in an EM program. For instance, our yeast and mold RODAC plates locations if determined by ISO 14644-4 where the formula is N = square root (A) where N is the minimum number of sampling locations (rounded up to a whole number) A is the area of the clean room or zone in meters^2.

Currently I take one swab per room and run for objectionables but I think I'm taking too few samples per room. Just looking for a document that helps determine the correct number.

A1: If you are taking swab samples in your EM program and looking for specific objectionable microorganisms, it would appear as though someone is confusing EM and MLT and combining the intent behind the two assays.

Why are you searching for a specific microorganism with an individual swab? Did you lose a bet?

All kidding aside, there appears to be a lack of rationale supporting your monitoring program.

You can refer to USP chapter 1115 for guidance. Please don’t be alarmed when you do not come across a mathematical recommendation for number of samples.

A2: The term objectionable microorganisms applies to non-sterile products. It has no meaning in sterile product manufacturing.

A3: I wouldn't apply ISO14644 for defining your viable EM program. Also just check your references within your documentation. Part 1 is for Classification of air cleanliness by particle concentration and previously referenced the calculation you mention, however this was updated in 2015 to a representative look-up table for floor area and provided you with the number of particle monitoring locations.

Refer to USP chapters:
<1115> BIOBURDEN CONTROL OF NONSTERILE DRUG SUBSTANCES AND PRODUCTS
Or <1116> MICROBIOLOGICAL CONTROL AND MONITORING OF ASEPTIC PROCESSING ENVIRONMENTS

I'm not sure what you are classifying/grading your cleanrooms as, however you may be over monitoring within some areas/rooms if you are applying the calculation to define the number of location you take. If you designed your monitoring plan based on risk and you could also review your historical data obtained from your established locations, then you may be able to remove some of them easily if they are not providing meaningful counts. Ensuring that your plan has sampling performed at the more critical areas along with locations to assess general room conditions etc, rather than for example 4 or 5 within a low risk room just because that's what your calculation stated is more appropriate. With regard to swabbing specifically you may find that you don't need these or at least in not in every room. depends on what and why you are swabbing.

A4: It occurs to me that your EM program may have been set up by someone with a nutritional supplement or food background. Those are the only areas I am aware of where there is a specific requirement to collect swab samples to detect objectionable organisms. Can you share what type of swabs you are using and what organisms you are screening for?

I agree with other responses that it is not appropriate to use a formula to determine number of sample sites for surfaces. You need to assess each area individually and select sites using a risk-based approach.

Purified water from a small unit for pharmaceutical preparations

We plan to have a small Elga unit within a cleanroom that produces purified water used for product formulation. This is for small scale manufacture to cover phase I to III/marketed.

Do we still need to follow the same principles as if it was a large scale water generation and distribution system and apply the same PQ phases of monitoring and intensity for the single water supply point? have a full year PQ? include and assessment of sanitisation etc?

Or can we perform a smaller equipment qualification with several days monitoring followed by a weekly/ 2 weekly sample?

Pour-Plate vs Membrane filtration for Bioburden analysis of UPB

Are there any papers or studies showing that Pour-plate is a better option compared to membrane filtration for Bioburden analysis of UPB? I saw some claims that the use of the cell suspension is more representative than supernatant in this case because the cell suspension can have more material that can block the membrane.

I know a study comparing both and showing the advantages is the way to go but I wanted to scan for some literature or white papers.

A1: What is UPB? Unpaid principal balance, union program board, etc.?

A2: Just google searched for UPB, it is defined as

Unprocessed bulk *(UPB) *is the material collected directly from the bioreactor in which genetically engineered cells grow and produce products. Low levels of adventitious agents such as bacteria, yeast, fungi, molds, mycoplasma and viruses that bypass detection during raw materials testing, may grow to detectable levels under the highly enriching conditions of the bioreactor. At this point in the manufacturing process, it is optimal to test for these adventitious agents and testing of each lot of UPB is a regulatory requirement.

Reference : https://www.eurofins.com/biopharma-services/product-testing/services/biopharma-product-testing-services/quality-control/lot-release-testing/

A3: I believe that UPB is an abbreviation for umbilical placenta blood. However, I would like to hear from Ricardo for confirmation of this abbreviation. I can see as to why the pour plate analysis would be a better option than membrane filtration due to the presence of cellular debris if UPB is umbilical placenta blood.

A4: Calling it cord blood would have done it for me.

In many instances, the use of acronyms does not aid communication.

A5: Sorry All for the confusion. UPB is the abbreviation for Bulk Drug Substance for a biologic.

A6: In general, membrane filtration is better because you can use more sample and rinse away any inhibition. That being said, with many matrices, you're correct, they can clog the filter, causing firms to use multiple filters for one sample which isn't ideal. We have successfully used a mammalian cell lysis buffer to lyse the cells and filter the product in some circumstances. If you only need to use 1mL of the UPB and you don't have any anti-microbial properties in the product, a pour plate is probably about the same as membrane filtration. If you can use more sample to get higher sensitivity and it can be filtered with the help of a dilution, heat or cell lysis, membrane filtration would be better. A pour plate is definitely more manual and requires the media to be a specific temperature so as to be molten but not too hot that it will destroy organisms so there is more of a "classical" microbiology aspect to pour plates as well.

A7: Perhaps you mean unprocessed bulk sampled from a biofermenter or discharged to a holding tank that will contain the cells that produce a monoclonal antibody. It does not become a bulk drug substances to later in the purification process.

The question may be should you spin down the cells, i.e., mammalian cells, yeast or bacteria and test the supernatant for adventitious viruses, mycoplasma, bioburden or sterility or lyse the cell prior to testing. For bioburden, testing the media or a supernatant of the cell suspension should be adequate using either a membrane filtration or plate count method. With a larger sample size cell present in the sample may result in clogging.

Remember, the downstream processing is not a sterile operation but the bioburden is controlled by the actual process steps, bioburden-reduction filtration, viral inactivation, time limitation, and refrigeration temperatures.

HVAC validation

I am working in tablet and capsule formation plant.

I have Question regarding to HVAC validation. During HVAC validation, where should we do viable monitoring by Settle plates?

Is possible to do viable monitoring before test completion of Particulate counts?

Actually company has done air velocity and filter integrity test but particulate count test is pending. In between this, my Environment monitoring by Settle plates schedule is due.
What should I do to planned environment monitoring ? Can i go for environment monitoring before particulate count?

A1: The microbiological monitoring must in any case be compliant by construction characteristics and physical controls suitable for the class being monitored.
You talk about the production of solids, but what is in the qualification phase is important, to know if you are in an ISO classified environment (or Annex 1) and therefore you must carry out the checks required by the corresponding environmental class. The correspondence must be for both physical and microbiological characteristics.
If, on the other hand, the place is not subject to classification and your controls are the result of a risk assessment, the parameters are less stringent and you can adapt them to what is useful for your production typology.

A2: Yes. These results can be generated concurrently using bio-fluorescent particle monitoring methods during HVAC and HEPA filter qualification for non-sterile manufacturing facilities. A question would be what is the appropriate acceptance criteria if the facility is not classified to an air cleanliness standard. Perhaps as a default you could use the ISO 8 (Grade D) standards.

I believe it is time for pharmaceutical companies to request that contractors who connect cleanroom qualification and period recertifications use these emerging technologies and have the instrumentation available.

A3: Manufacturing plant is classified area: ISO 8.

Our manufacturing plant is maintained as per air cleanliness classes and company scheduled every year "Requalification of HVAC validation". In this program, "Air velocity test and Filter integrity test" of HEPA has been done in ISO 8 area but "Particle count test" is not still done.

Should I go for "Environment Monitoring" without completion of Particles test?

Or Should I wait for first to complete Particle test and then after go for EM by Settle plates.

A4: Based on this additional information, because this is a routine requalification, not a new facility, I would say go ahead with your viable monitoring as scheduled.

A5: Is any guideline or article available for requalification of HVAC system validation?

A6: I would recommend obtaining documents from the American Society of Heating and Air-Conditioning Engineers (ASHRAE) that address the design, qualification, and operation of HVAC systems.

EM Trends and setting limits in nonsterile manufacturing

Assume a situation where a nonsterile manufacturing firm has performed EM trending most recently, three years ago. The current report being written contains the last four years of data. Action and Alerts haven't been adjusted for three years. With no specification for nonsterile environments, what is the best method to go about determining appropriate action and alert levels? From both a statistical standpoint, and from the standpoint of "is data four years old truly relevant to limits that would be set at the present time?"

A1: The critical parameter is the number of data points not necessarily the time period. If you have 300 data points it should be solid.

The first step is to plot the data to determine if it is normally distributed. If so you could use the 95% percentile (alert level) and the 99% percentile (action level).

I would recommend using a non-parametric 95 and 99% confidence limit approach. As it is not practical to have different alert and action levels for different rooms in the facility, if the rooms are statistically different use a single acceptance level across the facility. If one or more rooms are statistically different investigate why. For example, poor HVAC system performance or high operator number of activity, and correct it.

A2: Thank you so much [name redacted] for this approach. I will be crunching numbers tomorrow.

I was thinking perhaps there should be different limits for mold recovered in EM? What are your thoughts on that?

A3: No. Monitoring for total aerobic microbial count includes bacteria, yeast and mold.

However, if you consistently isolate mold the facility may be compromised due to water damage.

A4: I assume with 4-years of data you would have sufficient data to perform a statistical analysis of the results to see how the data are distributed. Although there is no specific right or wrong, what I have seen and working with is using the 95% cut-off for alert levels and 99% for action levels. The benefit of this method is that the data don't need to be in a "normal distribution". Depending on the criticality of the area / location of EM samples you can decide if some parts of the facility would require tighter alert and action levels. Use the data set of the specific area to calculate the alert & action levels separately then. Always apply a risk based approach with this and tailor the program and levels to the outcome.
One other thing to take into account besides alert and action levels is the presence of micro-organisms of concern. This depends on the type of product and route of administration and need to be established as well.

A5: What we have found in our non-sterile manufacturing facility is that high mold counts we see is primarily from the cardboard. We did a study and found the same types of molds at the species level in both the cardboard and facility air.

A6: I would consider the presence of cardboard packaging and wooden pallets in manufacturing areas to be a cGMP violation and practices that would have been discontinued years ago.

I and others published multiple articles on this issue.

Cundell, A. M. (Task Force Member) 2,4, 6 Tribromoanisole and 2,4,6 Trichloroanisole- A Review of Tints and Odors in the Pharmaceutical and Consumer Healthcare Industries. Pharm. Technol. September, 2012 pp56-62

Cundell, T. Mould Contamination in Pharmaceutical Drug Products and Medical Devices. Eur. Pharm. Rev. 18 (6): 1-18 2013

Cundell, T. Mold Monitoring and Control in Pharmaceutical Manufacturing Areas. Amer. Pharm. Rev. 19(5):10-19 July/August 2016

*Cundell, T. Fungal Outbreaks Associated with Pharmaceutical Drug Products and Medical Devices In **Fungi: A Handbook for Life Science Manufacturers and Researchers**Jeanne Moldenhauer (editor) Horwood/PDA 2019*

A7: I have found the same thing in which cardboard was the source of air borne mold in a non-sterile manufacturing. Cardboard containers are often used to store packaging components before placement on a filling line. In addition, cardboard was also a source of mold contamination in refrigerate rooms that stored micro. supplies due to water condensation that had occurred in the room. However, the mostly likely source of mold contamination is due to mold growing in the air handling system of a non-sterile manufacturing plant due to a poor design. The second likely source is that the pre-filters of an air handling system was either not present or compromised which allowed dust containing mold spores to enter the facility. In conclusion, there are many sources of mold contamination in a non-sterile manufacturing facility because the facility is not in compliance with ISO 8 requirements.

A8: Even if it is not exactly the subject of the initial question, I focus on the problem related to cartons and molds.

Years ago I found, by checking the bioburden of empty aluminum tubes, a high number of molds. By carrying out the investigations it was understood that, since the tubes were positioned open inside the packaging, despite the fact that there was a waterproof paper for protection between the tube and the cardboard, the molds came from the cardboard. Even after gamma-ray sterilization the problem recurred as the carton was reinfected with molds during unprotected storage.

A9: Manufacturers of packaging components are aware of environmental control to prevent particulate contamination and your company should emphasize these environmental controls during vendor audits. Component packaging should maintain the sterility of irradiated components and prevent particulate contamination. For example, glass vials should be shrink wrapped during shipment to prevent glass-to-glass damage and cardboard sourced contamination.

Maximum Room Capacity Challenges

Historically our facility has always challenged a maximum room capacity during EM PQs, APS runs, or for yearly particle reclassification efforts for each cleanroom. However, I was wondering if anyone knows if there is any regulatory guidance specifically calling for maximum room capacity challenges? Our argument has always been that in order to show that there is no product impact it's important to show that the dirtiest things in the room (i.e. people) will not exceed a certain capacity and that capacity is challenged periodically, but if there is specific guidance I want to also ensure we are referencing that.

A1: As far as guidance references to personnel during Aseptic Process Simulations, the FDA aseptic process guidance includes "Number of personnel and their activities" in the list of issues to consider for

worst-case conditions in Process Simulations Study Design. It also includes "maximum number of personnel present and elevated activity level" under Environmental Conditions.
These are the easy ones to find. I know there are others, but don't have time right now to look them up.

A2: FDA's Guidance For Industry document Sterile Drug Products Produced by Aseptic Processing , Chapter IX. Validation of Aseptic Processing and Sterilisation, section 6 "Environmental Condition" describes :

To the extent standard operating procedures permit stressful conditions (e.g., maximum number of personnel present and elevated activity level), it is important that media fills include analogous challenges to support the validity of these studies.

A3: The proposed revision to EU Annex 1 requires that:

Clean room classification should be carried out in the "at rest" and "in operation" states.

i. The definition of "at rest" state is the condition whereby the installation of all the utilities is complete including any functioning HVAC, with the main manufacturing equipment installed as specified and standing by for operation, without personnel in the room.

ii. The definition of "in operation" state is the condition where the installation of the cleanroom is complete, the HVAC system fully operational, equipment installed and functioning in the manufacturer's defined operating mode with the maximum number of personnel present performing or simulating routine operational work. In operation classification may be performed during simulated operations or during aseptic process simulations (where worst case simulation is required).

iii. The particulate limits given in Table 1 above for the "at rest" state should be achieved after a "clean up" period on completion of operations. The "clean up" period should be determined during the classification of the rooms (guidance value of 15 to 20 minutes).

Requesting that the worst case scenario be simulated with the maximum number of personnel the space will 'normally' anticipate for operations.

A4: As per your requirement, statements copied below for your ready reference.

ISO 14044 Cleanrooms and associated controlled environments — Part 1: Classification of air cleanliness

2.4 Occupancy states
2.4.1 as-built
condition where the installation is complete with all services connected and functioning but with no production equipment, materials, or personnel present
2.4.2 at-rest
condition where the installation is complete with equipment installed and operating in a manner agreed upon by the customer and supplier, but with no personnel present
2.4.3 operational
condition where the installation is functioning in the specified manner, with the specified number of personnel present and working in the manner agreed upon.

3.1 Occupancy state(s)
The particulate cleanliness of air in a cleanroom or clean zone shall be defined in one or more of three occupancy states, viz. “as-built”, “at-rest”, or “operational” (see 2.4).
NOTE It should be recognized that the “as-built” state is applicable to newly completed or newly modified cleanrooms or clean zones. Once testing in the “as-built” state is completed, further testing for compliance will be performed in the “at-rest” or the “operational” state, or both.

Yeast in purified water-non sterile pharmaceutical

How do we address the yeast (RG2) organism found in Purified water sampling point?
Appeared second time in two years and on the sample point that is only used for sampling.

A1: Rhodotorula glutinis RG2 is a common environmental yeast that is viewed as an emergent pathogen especially with immuno-compromised people. However, it is unlikely to be found in purified water that would not have the sufficient nutritional level to support the yeast.

Types of strains

I would like to take a doubt with you about the strains.

I currently buy second passage from reference ATCC certified strains for the control of culture media and tests of suitability to methods (validation), but due to the high price of the dollar I am having trouble acquiring these strains that are very expensive.
I saw that some suppliers sell first or second passage from reference control strains NCTC / NCTF and I would like to know if there are any regulatory impediments to the use of these control strains in my tests.
Or if I really have to use ATCC certified strains.

A1: EP, harmonized with USP and JP, says this:

- Staphylococcus aureus ATCC 6538 NCIMB 9518 CIP 4.83 NBRC 13276
- Pseudomonas aeruginosa ATCC 9027 NCIMB 8626 CIP 82.118 NBRC 13275
- Bacillus subtilis ATCC 6633 NCIMB 8054 CIP 52.62 NBRC 3134
- Candida albicans ATCC 10231 NCPF 3179 IP 48.72 NBRC 1594
- Aspergillus brasiliensis ATCC 16404 IMI 149007 IP 1431.83 NBRC 9455

So you can buy from the organization indicated in the pharmacopoeias.

A2: I would draw your attention to a PDA Journal article I co-authored on this topic:

Cundell, A. M. S. Chatellier P. Schumann, and R. Lilischkis. Equivalence of Quality Control Strains of Microorganisms Used in the Compendial Microbiological Tests: Are National Culture Collection Strains Identical? PDA J. Pharm. Sci & Technol. 64(2):137-155 2010

A3: I would like to know if I can use control strains instead of certified strains.

I have a supplier that sells the same NCTC / NCTF strain, in two types: control and certified. So I would like to know if I can use the control strain.

The supplier said I can use it, but I would like to have some evidence that there are no problems in using them in microbiological Quality Control.

Purified Water sampling frequencies

Kindly update on the frequencies for purified water samples.

Is it any criteria or guidance which states that return loop sampling point have to sample and analyse on daily basis?

A1: As per PDA TR 13, Appendix A: Current Guidelines for Typical Environment Monitoring Frequencies and Levels- Water

Purified water: Monitor distribution system daily when in production.

Water for Injection:Rotate testing at all points weekly for micro, test return loop daily for chemistry and Endotoxin. Test feed water to daily.

Moreover refer USP <1231> 6.4 Routine sampling plan,recommends sampling frequencies vary from daily to monthly.

In general to demonstrate the state of control in water system sampling plan should include at least return loop, generation samples on daily basis.

A2: Considering the worst case scenario and the scientific rationale PW should be sampled on daily basis.

Also refer the guidelines mentioned by [name redacted].

A3: I think PDA TR-13 is speaks about Environmental monitoring and in USP 1231 no where mentioned about the sampling frequency of generation loop. If you don't mind, can you share me those documents because in our plant I am fix the frequency of sampling.

A4: I have been following this discussion. As far as a general recommended sampling frequency for purified water systems, you will find none. I can see a purified water system sampling frequency at one week intervals or even 2 or 3 times a week. Sampling frequencies of a purified water system is generally determined during and after the operational qualification of the purified water system. They generally fall within a range as what is indicated in USP 1231. Without knowing the design, system components that are present in your purified system and operational validation data that had been generated, it would be difficult to make any recommendation for determining a sampling frequency based upon a conducted risk assessment without having this information. Let's say that you had a successful operational and performance qualification of your purified water system, I would also have a lot of questions for justification as to why you are taking daily purified water samples for microbial analysis. For example, is the system properly designed and are you maintaining the purified water system (e.g., by having proper water flow rates, changing of ultraviolet light bulbs and filter as required, is there the presence of proper microbial control measures (UV, filtration, ozone or heat) within the system, etc.)? From an auditing perspective, the sampling frequency of a purified water system would be one of my main points of investigation besides looking at the generated chemical and microbial test data for the distribution part of the system.

I do have a concern about the taking of water samples in the generation portion of the purified water system. I can see the taking of water samples in the different parts of the generation portion of the purified water system to see how each component is working only for informational purposes, but not on a routine basis.

However, I do have objections in the taking of routine microbial test samples in this generation portion of the purified water system. It is well known that high microbial levels would often be present in these sampling points such as after the multi-filter, carbon filter, and cation/anion exchange beds. Also, it would not be unusual to find Pseudomonas species and Pseudomonas related species (e.g.; Burkholderia, Ralstonia, Sphingomonas, and etc.) at these sampling points. Furthermore, it is not unusual to see the presence of microbial control measures such as Ultraviolet lights and 0.22- or 0.45-micron filters to be present to control the microbial content of the water if the system had been properly designed after these system components. I will admit that I have seen a lot of poorly designed purified water systems in the world, but I have also seen some very good ones. What do you gain in taking microbial samples in the generation portion of a purified water system on a routine basis?

A5: Filtration of PW or WFI is not recommended as it is a source of potential contamination.

A6: Please provide more information concerning your statement that filtration can cause contamination of PW or WFI systems. I do not understand your statement. It is very common to use filters to control the microbial bioburden in the distribution piping of purified water systems.

Problems do develop when filters for a water system are not changed on a periodic basis in which microorganisms will eventually be able to penetrate the filter by such mechanisms as growth thru and blow thru. If the filters are changed on a periodic basis, this will not occur. In addition, it helps to have differential pressure gauges on the filter housing to see when the pressure across the filter changes to indicate the presence of a problem with the filter.

A7: [name redacted], I was recently challenged by an agency inspector regarding PW sampling frequency. Yes, FDA is often wrong... But it seemed like the expectation from the agency was sampling on each day of production at minimum. Question was “You’re manufacturing 7 days a week, but sampling on 5 of those. How do you know the quality of the PW on weekend days, when production is occurring?”

Again, FDA is not infallible, but this seemed like a current expectation.

A8: Yes, filters in the pre-treatment system can have high bioburden counts. The benefit of sampling in the pre-treatment portion is the monitor the health of the system upstream so you can address bioburden problems before they move downstream creating biofilm issues in your loop. It can inform when the filter beds need to be sanitized or back washed.

With response to sampling for information only, that is typically frowned upon by regulators. Set your alert and action levels based on historical data. Address excursions so that your loop is not eventually impacted. I thought that sampling of pre-treatment systems was mentioned in the revision of <1231> in a recent revision.

A9: Filtration of PW was used in the past but it was common to find high counts even the filters were changed periodically because wet filters increase the probability of higher bioburden. Currently the best approach Is ozone/UV in a cool system or heat (70 Celsius degrees as a minimum). Personally I recommend ozone during no working hours, UV to inactivate ozone during working hours in the loop and ozone 24 hours in the tank.

A10: You could use the ISPE baseline guide for water and steam sampling and testing as a justification for your sampling procedure. Not sure whether they will accept it, but ISPE does not require sampling every day.

The real question is what type of product you are making and whether the water is the raw material in the product. If so, you also need to understand whether you committed to bioburden data for each batch of the water used as the raw material.

A11: Can u share with us the ISPE Guide u r referring. It’s a Paid Link. Available only for ISPE Members.

A12: The ISPE baseline guides are here. https://www2.ispe.org/imis/ISPE/Store/Category_Search_Results.aspx?InitialText=BASELINE. You can find one on water and steam systems on this page.

The ISPE Good Practice Guides are here, this is where the sampling guide is. https://www2.ispe.org/imis/ISPE/Store/Category_Search_Results.aspx?InitialText=ISPEGPG You can find the sampling guide on this page.

All ISPE docs are available to anyone, albeit at a premium if you are not an ISPE member. But all can be purchased from ISPE by anyone.

You may also want to connect with the "water engineers" or other water specialists in your company - they may have these docs already.

Filling in Grade A isolator: holding tanks and filtrations of products

I'd like to ask your opinions about manufacturing and filling of aseptic and terminally sterilized products. Production would take place in Grade C manufacturing facilities and in Grade A filling isolator. Manufacturing and filling take place on different working days.

1) After manufacturing phase is filtration from manufacturing tank to separate holding tank required for aseptic=non-terminally sterilized products? After the holding tank there would be sterilizing filtration before filling. In this case, is it so that bioburden sample is taken from the holding tank, as sterilizing filtration takes place after that?

2) Or could the solution be (double) filtrated directly from the manufacturing tank to filling? In this case, is bioburden sample taken from the line after the first redundant filter and before sterilizing filter at the latest possible time point before filling is ended? Or is the bioburden sample taken from the manufacturing tank before the filtrations?

3) What about terminally sterilized products, do you have the holding tank and two-phase filtrations before filling? Or do you filtrate the product directly from the manufacturing tank to filling?

Personnel locks, Grade C area surrounding Grade A filling isolator

Can you help me with designing personnel locks? What kind of personnel locks do you have leading to Grace C area (manufacturing and filling of aseptic and terminally sterilized products)? Production would then take place in Grade C manufacturing facilities and in Grade A filling isolator.

As grade A filling isolator should locate in grade C facilities, personnel lock is divided in Grade D and C areas.

1) Do you have separate personnel locks for entering to and leaving from Grade C facilities? Or only one lock where personnel flow for both directions is allowed?

2) Do you have step over benches to divide Grace D/C parts of the personnel lock or door between these two cleanliness areas?

A1: Use access control with the area that you're working on.

Sterility for rapid method

I want to know what rapid method could give me a low risk to release a sterile product with a low risk of fail, i'm not going to stop using sterility, but i want a method to reduce risk and time.

Anyone could tell me their experiences.

A1: Without knowing more details, there are many options that could suit your needs. Methods that utilize ATP, respiration and direct cellular counting/fluorescence are all great methods (depending on many factors).

This should get you started: https://rapidmicromethods.com/files/matrix.php

In short, it depends.

A2: CORRECTION to the link (not https): http://rapidmicromethods.com/files/matrix.php

A3: There are a number of technologies that have been successfully validated for sterility testing. The choice of system will be based on your user requirements, such as time to result, compatibility with your test sample, level of sensitivity, etc. Unless one knows the user requirements it is difficult to make any recommendations. As Mark stated in his reply, "it depends."

As for “low risk of fail,” no one should use an alternative or rapid method unless it has been fully validated. After this, the risk of failure should be non-existent, unless the sample is contaminated.

Sample size per batch for USP 61 and USP 62 testing

Is there a regulatory requirement that defines the sample size per batch of finished product for microbial enumeration testing ? How many samples shall be tested from a batch ? Samples taken from beginning, middle and end ensure that random sampling is representative of all time points. But how do we decide on the number?

A1: Based on batch size, you can calculate sample size using n=Square root of N+1

N= batch size
n= sample size

A2: Does anyone know the origin of square of n+1? Was there any risk assessment completed that resulted in this?

A3: I believe the industry practice is to take a 10 g sample dilute in 90 mL of diluent that may contain neutralizing agents, i.e., Lecithin and Polysorbate 80 and testing 1 mL, i.e., 1 g of product. This is implied in USP <61> and <62>.

The question of batch homogeneity comes up for both release testing and the collection of stability samples. For release testing a bulk sample may be used when justified while the stability material must be in the primary packaging and can be selected randomly from the lot.

For a validated product any sample should be representative of the batch.

Ditch the idea of the square root of n plus 1 sampling plan.Unnecessary and not statistically sound.

A4: As you stated sample for Finished product, single representative sample of respective batch is enough and QC/Microbiology has to confirm that required samples quantity for complete microbiology related testings.

A5: Here is a paper that describes the origin of the square root of (n+1) statistical sampling scheme: https://www.pharmtech.com/view/statistical-solutions-square-root-n-1-sampling-plan.

A6: [name redacted] thanks for the reference.

What is the batch size?

For a batch of compressed tablets would be the number of individual tablets, the number of filled containers, or the number of pallets?

A7: The alternative to grid based or proportional sampling is risk based and process based. It's easier to set a simple standard for those unfamiliar with the rings of control. But I think this is the same shift in the industry towards risk based models using QRM principles (see Annex 1 updates etc.)

Use of BCSA

If a sample shows microbial presence via ATP activity, should the sample be streaked on both non selective agar as well as BCSA? I believe USP indicates non-selective only is sufficient..

If a sample does not show ATP activity, am I correctly assuming that subtypes of B cepacia would not fly under that radar, and additional streaking on BCSA is not necessary? This bug is just fascinating isn't it?

A Celsis unit is used in this first step, as FYI.

A1: If a sample enrichment shows positive on ATP testing, that same enrichment must be streaked on Selective, BCSA and TSA. Same day, one must also complete the total count test so all the results come out in 72 hours- if the sample had recovery of non objectionables and/or the total counts were within specification limits.

Many companies discard the original enrichment and retest the product- this is cheating out of the initial test and take a chance that the preservatives would have kicked in and/or the contamination was uneven and they will pass the second test. This practice has just not been caught by smart inspectors experienced In ATP testing so far but just the matter of time.

A2: Think you'd need to validate your application of Celsis vs. the complex.

A3: I assume you are enriching your sample in a non-selective broth (e.g., TSB) prior to performing a qualitative assay in the Celsis? If yes, then you can streak the broth directly onto BCSA. This is similar to the BCC enrichment and plating strategy outlined in USP 60. However, USP 60 does specify specific incubation parameters in the enrichment broth prior to plating, so I would check on this first.

To answer your question regarding a negative result in the Celsis, and whether you would still detect BCC (or any other organism), you would have to validate that low levels of organisms would be detected using the enrichment step prior to Celsis, and also that non-contaminated samples would not elicit a positive result in the Celsis (under the established bioluminescence background noise, usually NMT 3X the baseline for the Celsis system).

A4: If you get a positive for an ATP bioluminescence enrichment broth, I would instead streak an aliquot of the positive broth onto TSA Petri dish to confirm whether the positive ATP microbial test result was due to either a sample having an high ATP level or it was from the presence of a microbial contaminant. I would perform a biochemical identification of the recovered isolate. At this point, you do not know whether the microbial contaminant came from the sample or was introduced during the conductance of the ATP test. It is my opinion that there is no need to use selective agars at this point since the ATP bioluminescence test method is not a USP method. If a microbial contaminant is indeed isolated from the ATP bioluminescence enrichment broth, I would perform the methodology of USP Chapters 60, 61, and 63 to confirm the initial ATP test result and determine the level of the contaminants present before making a decision as to whether passing or failing the test sample for microbial content. It is best to use ATP bioluminescence test as a screening tool to determine if microorganisms are or are not present in a sample. No ATP positive results, the batch can be released.

A5: I agree with your comment. No matter what rapid microbial test method that is being used for microbial content testing of non- sterile products, I think that it would be wise to use the three indicated Burkholderia cepacia complex species in USP Chapter 60 as part of your validation test organisms for aqueous product for the detection of microbial contaminants in order to demonstrate equivalence of the Celsius ATP Bioluminescence test methodology to USP Chapters 60, 61and 62.

A6: When you validated you ATP methodology, did you develop any analysis for routinely checking new media, and other products that could be autofluorescent prior to using it as a test method? Determination of whether substances could yield false positives is an important part of validation.

A7: What a great collection of info, and some great questions, everyone. Thank you much for that. As I don't know where to begin to respond, I'll start by throwing this large rock in the pond:

For our USP purified water testing, our enrichment step uses the Alternative enrichment method from the Water / Wastewater Manual (the exact name slips my mind) but it is mentioned specifically in the USP as

acceptable. That enrichment step calls for the use of LTB (lauryl tryptose broth) as well as LES Endo. An auditor noted that we are using LTB and immediately exclaimed “that’s not in the USP!”. I suppose indirectly it is, though... However, our method validation revisions over the years (not 2-4 years, but 20..) wasnt the most keen on exactly checking for changes in that Alternative enrichment step. The older versions called for use of m-Endo, and “an endo-type medium” (going from memory here..), where the newer versions call for LES Endo (as replacement for the “endo-type medium”..) The formulations of m-Endo and LES Endo are significantly different. At least different enough to say it’s just not the same media. As I was doing the line by line comparisons between old versions of the Alt Enrichment and current, I began to question the value added to all of this. Why bother even using an Alternative Enrichment? This was a bit taxing but I laid out the facts in the end. (As a disclaimer I’ve been helping oversee the lab for about a month or two on a contract basis)

But I believe [name redacted] (I believe.... or [name redacted]) mentioned that there really is no use anymore in using selective agar. Would the recommendation be to change our method to USP Proper? Meaning, not straying off course with the Alternative method? That alternative method does appear at least, to be more robust in the detection of coliforms, but I’ve seen no data to date that supports that. And not sure how much that matters in the end...

Excellent points [names redacted]. There were good questions in there that I still need to consider. Especially [name redacted] point about being on top of false positives. I had not thought of that, to be honest.

A8: An ATP rapid method MUST be validated for its intended use and that includes method suitability to check for false positives and negatives. Therefore, a positive result does not need to be confirmed.

Also, if the rapid method is being used as an alternative to USP 60 (or any other compendial test), the ATP method must be validated as being equivalent or non-inferior to the compendial assay.

Finally, using a validated ATP method does not require any confirmation of initial results. This is why we validate the rapid method in the first place.

ATP systems can be used as screens but can also be used as an alternative to the current or compendial method.

A9: For sterility testing, you are correct that a positive ATP bioluminescence test result does not need to be confirmed. However, microbial limits testing is a different ball game in comparison. The reason that it is a different ball game is that you are allowed to have up to a certain numerical microbial limit in non-sterile product formulations. You may have a positive ATP bioluminescence test result when you have an acceptable microbial count for a non—sterile product formulation. For this reason, I would recommend that an ATP test method be used as a screening tool for the presence or absence of microbial contamination. A confirmatory test needs to be perform whether the count for a positive ATP bioluminescence test result in order to determine whether the sample is within the acceptable microbial limits for a non-sterile product formulation. I’m not talking about the indicator organisms in USP chapters 60 and 62. If you have a positive ATP bioluminescence test result for the presence of an indicator organism an enrichment test sample, there is no need to confirm the test result. However, most of the detected organisms in an ATP bioluminescence enrichment broth is not one of the USP indicator organisms in USP chapters 60 and 62. By using selective/ differential agars for subculturing from a positive ATP bioluminescence test result, it is a waste of time and money when the isolate can be confirmed as a USP indicator organism by subculturing onto TSA for Gram-staining and phenotypic/ genotypic identification.

If a microbiologist at a non-sterile manufacturing facility did not confirm whether a positive ATP bioluminescence test result was within the acceptance criteria or not a USP indicator organism or an objectionable organism, they would not be employed for long and the company would drop the usage of the rapid microbial test method due to them losing money for rejecting acceptable finished product.

A10: Be aware the classic gold standards methods - are not. I invite [name redacted] experience - but mine has found alternative method showing better recovery than 61/62.
Selective media may be driven by protocol but even in cosmetic and household product applications, mixed cultures are rare, and validation based on clinical isolates leaves the question in my mind of enviromentals. Hang around long enough, folks will find aeruginosa's that are cetrimide sensitive (as in no growth on Cetrimide agar) and cepacia's triclosan senstive (as in no growth on PIA).

A11: This has been a recent theme with FDA inspectors; that unless you follow USP 60, including the media used for the enrichment step, it is questionable whether or not you are recovering BCC in your test method.

That being said, if you have validated an alternative enrichment medium and/or incubation parameters, and you can show this data to the inspectors.

But if your alternative medium has not been properly validated as an alternative to what is currently expected to be used, then the easiest fix is to follow USP 60.

A12: My responses were based on the original poster's question, which was the use of a qualitative ATP method and the subsequent confirmation of the presence of BCC.

Now to your point regarding microbial limits testing. A quantitative ATP method should be used as an alternative to the compendial test, as you obtain a numerical value. When a qualitative ATP test is used, it is not possible to confirm the actual number of organisms in the original sample, unless you preformed a dilute-to-spec method (see my paper that describes this: xxx), which will only give you an estimation of cell count but cannot be used to compare with a limit or spec.

Even using a qualitative method as a screen has its limitations UNLESS you get a result that demonstrates there are no recovered organisms. In this case, you can conclude you are below your spec because there are literally no viable organisms in the sample. This was the strategy used by GSK Italy when they validated the PallChek qualitative ATP system (no longer available) for a non-sterile nasal spray back in 2004. If the ATP level was below the baseline, they concluded there were no organisms in the sample. But if they got a positive (no matter what the ATP level), they had to repeat using the compendial method to see if they were above or below the spec. Since the latter only occurred a few times a year, performing the repeat testing was not a big deal. Nd this strategy was approved by FDA as the very first PAT application using a rapid method.

Regarding your last statement, if a rapid method is properly validated, acceptable finished product should never be rejected. The notion that VALIDATED RMMs will give false positive results resulting in lost product is a misconception that has been bogging down acceptance in the industry for decades.

Suitability test/Aceton

Do we need to perform a suitability test for Aceton (cosmetic product for nail polishing)? if so what would be the best way to neutralize it?

A1: Same as a solvent-based product you should be able after a risk assessment forgo microbial testing.

A2: I don't think you can neutralize it but you could dilute it in water or probably Fluid A/D. Also, if you're performing membrane filtration, be sure you have a compatible filter as Acetone can dissolve certain filters. I'm not sure about the requirements to test nail polish remover.

A3: Yes Acetone. What is your argument not doing micro testing nor suitability?

A4: The stuff is miscible with water but unless water is significantly in your raw material, yours is effectively an anhydrous product with intrinsic antimicrobial efficacy. https://www.sciencedirect.com/science/article/abs/pii/000293945690513X

A5: The argument would be that there is insignificant risk to your product or process and as Tony Cundell mentioned, a suitable risk assessment is the means of establishing this.
From what I was able to ascertain from the 1956 paper referenced in a previous post , the culture used was a non-spore-former, " Micrococcus aureus" (an outdated heterotypic synonym for Staphylococcus aureus it would appear). The results (at least for the Phenol Coefficient - I did not download the full article) indicated poor bactericidal properties of diluted acetone.
If anyone has accessed the complete article maybe they can share whether spore-formers / spore suspensions were tested and whether sporicidal activity was established.
In the absence of sporicidal activity, the risk of acetone would more than likely be akin to that of IPA e.g., incapable of supporting growth per se, the solvent if adulterated with spores during production or handling could act as a vehicle for post use / post evaporation cross contamination of surfaces and depending on the downstream product contact interfaces, the risk possible of outgrowth scenarios cannot be ruled out.
The Risk Assessment should encompass
1) The Material - Production & Handling conditions relevant to solvent manufacturer / distribution) and
2) Usage procedures, particularly where other products are being manufactured using shared production facilities .
The principal risk most likely relates to indirect contamination of other products that might support spore outgrowth.

A6: Think you guys are carried away with this. It is acetone for nail polish - hostuile and anhydrous in hostile and anhydrous. This should be a pro forma effort for the microbiologist, not a research project.

A7: Agree. It is a nail polish remover. It should not be a R&D project. For acetone nail polish removers, they are mixtures for the most part consisting of acetone and some water. There are some other minor ingredients such as fragrance and color that may be present if used. However, acetone is the major ingredient of these formulations. Acetone nail polish removers are generally not tested for microbial content because acetone is a solvent that is hostile to the survivability of microorganisms. You do not see organisms growing in acetone decolorizers for doing Gram-stains of bacteria. A conducted risk assessment would have indicated the hostile nature of this nail polish remover product without even conducting any microbial testing to support the justification of the conducted risk assessment.

A8: I think we are all on the same page here, no research project needed!

From a general interest perspective, I did subsequently come across a number of papers that indicated that Acetone has "no" significant sporicidal activity. Obviously, product, process & usage will determine the relevant risk.
Don't quite understand, how or by who the need for a "research project" was introduced into the discussion thread, though it seems it might have been mistakenly attributed to my recent post [06/11/21]. In it I did advocate a "suitable" Risk Assessment Process, to allow the user (poster) objectively determine the "Level of Effort" appropriate to their process, rather than relying on subjective "pro forma" efforts or going over the top.

A9: in context of nail polish and cosmetic grade RM's, any effort on acetone in this context would be a waste - including risk assessment and especially any testing.

A10: I'm in agreement with Derek that Acetone does not have any sporicidal activity. However, acetone does have bactericidal activity. From my perspective, conducting a research project in trying to validate the isolation of microorganisms from acetone would be just a waste of time.

As part of the risk assessment, I would look at how acetone is produced. From my understanding, acetone is produced directly or indirectly from propylene. Benzene is alkylated with propylene to produce cumene which is then oxidized to produce phenol and acetone. In addition to this cumene process, acetone can be produced in which there is the direct oxidation of propylene (Wacker-Hoechst process) or hydration of propylene to give 2-propanol which is then oxidized (dehydrogenated) to acetone. Based upon these chemical production processes and the starting materials, it is my opinion that it is highly unlikely that acetone would be contaminated with microorganisms.

In conclusion, I'm in agreement with Phil that it would be a waste of time in conducting suitability and microbial limits testing on acetone.

Let's hope in the future that no one ever gets the bright idea to conduct microbial suitability testing on acetone. Hopefully, they will see this discussion on the PMF as why not to conduct microbial testing on acetone.

A11: There's a saying that is possibly applicable to this that a great guy and friend, T.C. Soli told me (and I most likely have it wrong but I'll try paraphrasing):

"There's a reason you don't set out traps for tigers in North Carolina."

I think we sometimes lose sight of the rationale of our microbial testing - patient safety! For example - We shouldn't be testing for obligate thermophiles in cold purified water systems used for manufacturing non-sterile because honestly what difference would it make if they're there. Zero impact to patient safety.

in-house isolate and passage

How do you apply seed lot technique for in-house isolates, eg. EM isolates - what is considered passage 0; original microbe on an original EM plate, pure culture of this EM isolate or freeze-dried/deep frozen culture of this EM isolate?

A1: I have not seen passage rule applied to EM isolates. In my opinion, the EM isolates would change at each transfer from the original recovery plate to growth media- slowly losing their virulence as they get fed all the nutrients. Unless you decide to transfer them on the original media and freeze. In that case, first recovery on the EM plate would be considered T 0 in my opinion.

A2: I have one question regarding doing MLT test for Raw materials going to be used in oral liquid products.

Is it required to do MLT test for each raw material like API, excipients irrespective it is there in monograph or not ?? If yes what is rational, why it is required and if no why not needed.

USP <62> BTGN testing, maximum volume/dilution for EE Mossel transfer

For Bile-Tolerant Gram-Negative Testing per USP <62>, you would first prepare a dilution in SCDB using not less than 1 gram of product, incubate at 20-25, and then transfer to EE Mossel Broth. The chapter states: "Unless otherwise prescribed, use the volume corresponding to 1 g of the product, as prepared in Sample Preparation and Pre-Incubation, to inoculate Enterobacteria Enrichment Broth Mossel"

Typically we would make a 1:10 dilution and transfer 10 mL into 100 mL of EE Mossel Broth, but I was curious if there is a specific ratio of SCDB to EE Mossel that should not be exceeded, since <62> does not specify volumes. At what point would you be diluting the EE Mossel too much and disrupting the selective properties?

A1: Wouldn't a 1:10 dilution be 10ml into 90ml broth? Please correct me if I'm wrong here.

A2: The 1:10 dilution was referring to the initial dilution in SCDB. Usually 10 grams of product into 90 mL of broth. From there you would transfer a quantity to EE Mossel Broth.

A3: 10 mL of prepared sample after resuscitation the whole content should be transferred to 100 mL EE broth.

The proportion of EE broth and sample should be evaluated during method suitability.

10 mL of diluent used for sample preparation with culture should be transferred to 100 mL EE broth.If characteristic growth observed in EE broth and VRBGA the same volumes shall be used for routine testing.

In case of no growth in EE Broth if culture used is appropriate concentration and volume of EE broth should be adjusted accordingly (double strength EE broth etc.)

A4: You're correct. USP doesn't specify volume since testing carried out based on method validation recoveries.

BTGN testing is carried out by Qualitative and Quantitative methods. Basically 1g of product corresponding to 10ml (if transfer is done from 1:10 dilution) transfer is done into 100 ml EEB, Mossel for Qualitative test and for quantitative analysis we need to inoculate dilute quantities of product like 0.1,0.01 and 0.001 g in order to estimate the quantity of microorganisms present per gram or ml of the product.

In practice, all boiling media's such as EEB ,VRBGA etc should be freshly prepared for best results.

Capital 4 - Tamol

Recently, I've been made aware of a non-sterile product neutralization scheme for USP <61> and <62> that used TSB with CAP4. I've heard of CAP4 before, but don't recall if I've ever used it. In my attempts to look this up, it seems it could be made in house using a material called TAMOL SN. Our lab does its own media preparations, but before we go down this path, does anyone have any experience with this that they could share?

Growth Promotion of Media

I’m trying to find where it states that GP results need to be between 10 and 100. I found the indication in <61> where it specifies that the duplicate plate counts should not differ by a factor of 2. I recall when I was in the lab years ago, I had trouble getting bacillus to grow over 10 cfu and Kocuria (I’m aging myself here..) to overgrow (over 100). Is this a USP requirement in <61> that I’m simply not seeing?

A1: it's in <71> as part of sterility test requirements for media.

A2: Check USP<62>: "Inoculate a portion of the appropriate medium with a small number (not more than 100 cfu) of the appropriate microorganism.”

A3: USP <61> growth promotion section states:

Test each batch of ready-prepared medium and each batch of medium prepared either from dehydrated medium or from the ingredients described. Inoculate portions/plates of *Soybean–Casein Digest Broth* and *Soybean–Casein Digest Agar* with a small number (not more than 100 cfu) of the microorganisms indicated in Table 1 <https://online.uspnf.com/uspnf#C61-TB1>, using a separate portion/plate of medium for each. Inoculate plates of *Sabouraud Dextrose Agar* with a small number (not more than 100 cfu) of the microorganisms indicated in Table 1 <https://online.uspnf.com/uspnf#C61-TB1>, using a separate plate of medium for each. Incubate according to the conditions described in Table 1 <https://online.uspnf.com/uspnf#C61-TB1>. For solid media, growth obtained must not differ by a factor greater than 2 from the calculated value for a standardized inoculum.

In the microbiology labs I managed we targeted inoculums of 30-50 CFU to provide a good statistical number and to avoid exceeding the 100 CFU upper limit.

If you review the growth promotion data for commercially purchased media inoculums of 10 to 100 CFU, 10^1 to 10^2 CFU, 10^2 to 3 x 10^3 CFU and 10^3 to 10^4 CFU with quantitative and qualitative acceptance criteria. The media is used by a broad range of customers. This may be a source of confusion.

A4: The spec is less than 100 but if we had a culture that was close to zero, it may not show up and now we have a false negative result. Also, ISO 11133 does talk about a minimum of 10 CFU recovery from a <100 CFU inoculation for a valid result. I think it's one of those microbiology "truths" that don't technically have any regulation stating 10-100...but as microbiologists, we know if we get close to 1, we're getting close to zero when it comes to CFU.

A5: I'll check <71> again. I heard from an old mentor that the 10-100 might have been in the regs when I was literally in the hood validating a GPQ Program myself (old age / memory fades..) in 2006 or so. In any event, as a fun Saturday night follow up question, how would you rate the severity (I suppose risk to product..) of the practice of a GMP Microbiology Lab that regularly tests the Quality of drug and other regulated GMP products, not historically validating the GPQ Program in their lab per <61>, with regular GPQ testing not enumerating GPQ results (plates receive a pass / fail only), and in addition, the plant manufactures its own media?

How would you rate the severity, what are the implications, and what might have you come across in your careers in terms of either enforcement action, loss of customers, etc., due to something similar to this?

A6: More than 20 years ago there was an acceptance criteria of 10 to 100 CFU. Do not remember the reference.

A7: This is the current wording from USP
Sterility
Incubate portions of the media for 14 days. No growth of microorganisms occurs.
Growth Promotion Test of Aerobes, Anaerobes, and Fungi
Test each lot of ready-prepared medium and each batch of medium prepared either from dehydrated medium or from ingredients. Suitable strains of microorganisms are indicated in Table 1.
Inoculate portions of Fluid Thioglycollate Medium with a small number (not more than 100 cfu) of the following microorganisms, using a separate portion of medium for each of the following species of microorganism: Clostridium sporogenes, Pseudomonas aeruginosa, and Staphylococcus aureus.
♦Inoculate portions of alternative thioglycollate medium with a small number (not more than 100 cfu) of Clostridium sporogenes.♦ Inoculate portions of Soybean–Casein Digest Medium with a small number (not more than 100 cfu) of the following microorganisms, using a separate portion of medium for each of the following species of microorganism: Aspergillus brasiliensis, Bacillus subtilis, and Candida albicans.
Incubate for not more than 3 days in the case of bacteria and not more than 5 days in the case of fungi.
Seed lot culture maintenance techniques (seed-lot systems) are used so that the viable microorganisms used for inoculation are not more than five passages removed from the original master seed-lot.
The media are suitable if a clearly visible growth of the microorganisms occurs.

Looks like it has been updated. I also recently found out that the requirement for non-sterile product growth promotion acceptance criteria changed from 50-200%. Now USP<61> requires:
Growth Promotion of the Media
Test each batch of ready-prepared medium and each batch of medium prepared either from dehydrated medium or from the ingredients described.
Inoculate portions/plates of Soybean–Casein Digest Broth and Soybean–Casein Digest Agar with a small number (not more than 100 cfu) of the microorganisms indicated in Table 1, using a separate portion/plate of medium for each. Inoculate plates of Sabouraud Dextrose Agar with a small number (not more than 100 cfu) of the microorganisms indicated in Table 1, using a separate plate of medium for each. Incubate according to the conditions described in Table 1.
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.

And UPS <62> requires:
Test for Growth-Promoting Properties, Liquid Media
Inoculate a portion of the appropriate medium with a small number (not more than 100 cfu) of the appropriate microorganism. Incubate at the specified temperature for not more than the shortest period of time specified in the test. Clearly visible growth of the microorganism comparable to that previously obtained with a previously tested and approved batch of medium occurs.
Test for Growth-Promoting Properties, Solid Media
Perform Surface-Spread Method (see Plate-Count Methods under Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests 〈61〉), inoculating each plate with a small number (not more than 100 cfu) of the appropriate microorganism. Incubate at the specified temperature for not more than the shortest period of time specified in the test. Growth of the microorganism comparable to that previously obtained with a previously tested and approved batch of medium occurs.
Test for Inhibitory Properties, Liquid or Solid Media
Inoculate the appropriate medium with at least 100 cfu of the appropriate microorganism. Incubate at the specified temperature for not less than the longest period of time specified in the test. No growth of the test microorganism occurs.
Test for Indicative Properties
Perform Surface-Spread Method (see Plate-Count Methods under Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests 〈61〉), inoculating each plate with a small number (not more than 100 cfu) of the appropriate microorganism. Incubate at the specified temperature for a period of time within the range specified in the test. Colonies are comparable in appearance and indication reactions to those previously obtained with a previously tested and approved batch of medium.

A8: What would you say to the nonsterile firm that neither estimates or justifies the amount inoculated to begin with, does not inoculate two plates in order to compare at all, and simply assigns a “pass / fail” to the (single) plate without enumerating nor recording enumerated values - ever? And has never validated the lab / equipment / media per <61>?

A9:
USP 61 defines "a factor of two" for recovery so that's documented. They're certainly not following that with a qualitative "pass/fail". ISO 11133 also talks about 50-70% recovery rates based on general or selective media so that's another standard justifying quantification for solid agar media. My guess is that the customer was following what the vendor was supplying which is what many vendors did/do with their plate media: "Good growth"..."+++"...."Growth"...etc. Many vendors weren't/aren't putting quantifications on their C of A so it could be that your client took that as the standard...which it isn't for USP testing.

Certainly they'll get an observation for something like this and making their own media makes this worse. It's not that TSA and SDA vary so wildly in components (even though they're undefined), it's more about the autoclaving and preparation process. The quantification testing is there to ensure their process is robust and repeatable. I don't know about other vendors but our prepared and dehydrated media rarely fail in the hands of customers because of the quality systems we use to release the media. That's why I say it's not as much the media as it is the prep/sterilization/storage that needs validation and testing. USP 1117 is another document they should be familiar with, in addition to ISO 11133.

A10: I’m in agreement. A quandary that I’m facing is that I found this egregious practice completely on my own, in the midst of an agency audit, with evidence of cover up over the past 9 years at minimum. I’m not sure that the agency they caught it to this extent (a chemist and a generic manufacturing inspector that was quite green, showed up to inspect, and my faith in them personally has declined significantly over the past three months to be quite honest.)

A quandary, I’m facing here. No routine microbiological testing in the indefinite past from this particular manufacturing facility can be said to have validity. No methods were found (as in, to exist at all…) during the audit. Of all chem / analytical methods, only ONE was found to be legitimate. They never GP’ed per

<61> to qualify their media manufactured in house nor purchased. How do they begin to perform a suitable product impact assessment given this? I'd imagine there were multiple quality agreements (they are a CMO) among some top tier drug firms that were broken in the past decade or more, due to this alone.

I think my first step might need to be to remove myself from this situation completely. There are certain beds that dogs just simply need to lie in themselves.

I'm frankly disgusted with what I've seen. Inadequate oversight from our agency protectors.

A11: They are not complying with the requirement of less than 100 CFU particularly if they are inoculating into a liquid, you would have no idea of the inoculum level without verifying the count. Yes, you could count the colonies on the plate you inoculated but it is common practice to have a control plate(s) for the inoculum.

A12: Yes, they throw a quanti-loop (10,000 organisms minimum) into enrichment broth for a day, and generally just inoculate from that broth which is clearly 100 cfu. Sometimes they'll perform dilutions, sometimes not, neither of which is within an invalidated process. And No enumeration of plates in duplicate.

I'll know their intent when I see what immediate actions were taken in over a week, after my notification to them. This is just disgustingly egregious in my opinion. And I came across correspondence from 2012, a conscientious microbiologist pointing out to the lab manager at the time what <61> says about duplicate plating / averaging / etc. Response was more or less "uh, can we talk about that on the phone?". Whatever was spoken about on the phone, did not lead to the fixing of the method. This was knowing.

This is bad.

Microbial contamination in Povidine Iodine Products and anti tb samples

1. Can we perform microbial enumeration tests (Tamc & Tymc and specified pathogens) in povidone iodine products like solution, scrubs, gargles, ointments, cream. These are use for topical application. because iodine povidone has micobialcide and antiseptic properties. we perform test using inactivating agent many times and no growth was found. According to British Pharmacopeia it is clearly mention in oral solution, cream, topical aerrsol mongraph, microbial enumeration test should be perform to ensure the quality of products during manufacturing, storage and packaging.

2. We have received combination samples of anti tb tablets (Rifampicin + Isonizide+ Ethambutanol). These are antibiotics. Regulators want microbial enumeration test in tablets. According to British Pharmacopeia it is clearly mention in tablets monograph, microbial enumeration test should be perform to ensure the quality of products during manufacturing, storage and packaging. We have analyzed many samples of anti tb tablets but not found any growth. can we exempt this test for those products have antimicrobial activity.

A1: You need to first perform method validation at your end as per harmonized procedure. Refer USP 61 & 62 for details.

Later, if recovery is not working, u need to go for dilution method to get recovery based on specification of market.

A2: If the recovery is not obtained even after trying multiple methods, exemption is not an option. Still the product need to be tested with highest dilution or selecting one method which showed higher recovery of all methods tried during method validation. Instead of exemption, reduced testing can be done based on a risk assessment for respective product.

A3: Please recallPseudobacteremia Attributed to Contamination of Povidone-Iodine with Pseudomonas cepacia https://www.acpjournals.org/doi/abs/10.7326/0003-4819-95-1-32

Re: PW sampling frequencies - In Line / POU Filtration Sidebar

Just my tuppence worth on in-line filters,

I too always believed "in line" or POU (point of use) filtration / filters for PF / WFI systems (distribution system not generation) were frowned upon,
because ,
1) They would mask the true micro quality, or
2) Support biofouling at the upstream filter face and increase the risk of shedding with associated sporadic excursions, or
3) In the event of filter failure or poor maintenance intervention, could initally (before downstream mixing) produce a high level "contamination plug" that might not be detected. (Have actually seen this with bacterial spore contamination of WFI -post equipment service).

What's the current thinking?

MLT test

I have one query regarding performing MLT test for Raw materials like excipients and other raw materials.

If MLT is not part of monograph, is it required to do MLT as it is going to be consumed for liquid oral manufacturing??

If any suppose only chapter <61> is mentioned in monograph, is it required to perform Specified organisms as well or vice versa?

A1: In the view of my opinion when mlt test is mentioned in a particular monograph than it is madatory requirement, but if is not mentioned in monograph, than we go to particular general monograph as oral preparation, ointment, capsules, tablets etc., and accordingly in the usp it is clear mention perform mlt test. And in case of liquid oral liquid preparation water will be used so in aqueous preparation mlt is mandatory.

A2: I would suggest to perform MLT testing of raw materials if it is susceptible to microbial contamination as confirmed from supplier audit.

As you mentioned intended use for Liquid oral manufacturing ,I suggest to perform Microbial limit testing which shall cover enumeration (as per USP 1111) and pathogens(based on route of administration)in addition to that I would suggest to execute Burkholderia cepacia complex(as per USP 60).

If monograph describes either one of test ,I would suggest to complies as per monograph requirement.

A3: In conducting microbial limits testing of raw ingredients in which there is not a pharmacopeia monograph to perform microbial limits testing, it would be my recommendation that you conduct testing only on those raw ingredients that are susceptible to microbial contamination by performing a risk assessment. In general, a conducted risk assessment will tell you to conduct microbial limits testing on only those raw ingredients that are botanical in origin, earth derived (e.g., soil and mineral), and aqueous suspensions of raw ingredients that are either synthetic or natural that contain a preservative. You do not need to perform a supplier audit to make this determination. However, I would still perform cGMP audits of your raw ingredient suppliers.

As for microbial test specifications for microbial limit tested raw ingredients that are not monograph, this is an interesting good question. For example, it may be possible to have a raw ingredient that is used in

several different product categories of USP Section 1111. I have seen this to occur with many raw ingredients in which they are used in both aqueous topical and oral products.. The question is which microbial test specification do you use. You may have to generate your own internal microbial test specification for a raw ingredient that is susceptible to microbial contamination to cover each of the USP Section 1111 product categories that it is used in.

Sometimes in Microbiology, it is not that easy as to what the USP states in its guidance chapters if you are trying to apply finished product specifications of USP Section 1111 to raw ingredients.

Viable count monitoring in manufacturing area

We are performing viable count monitoring in manufacturing area by settle plate and active air sampling method at monthly frequency.

We have viable count monitoring log book for recording the raw data like plate exposure time, name of area, which activity is going on in that area at the time of viable count monitoring, who have done the Em, which media use for EM etc.

Manufacturing area is having separate area usage log for recording of their activities like area cleaning, which product is manufactured in that area etc . (Just like equipment usage log).

Please suggest that is it required to write the viable count monitoring activity in manufacturing area usage log also.

A1: Firstly, Monitoring is performed by microbiology division not by production unit. it is the responsibility of microbiologist to entry in separate log book of viable count in production area present in micro division. it is not necessary that entry should be in production log book as is not activity of production.

A2: Recording EM activity details in manufacturing area logbook isn't really necessary, since you can track your schedule activity from your schedule and TDS book, you can change the procedure with appropriate justification & find the reason 'why that documenting practice implemented' previously.

A3: As per my experience in some of the organisation QA will do, in some of the organisation production team will do the EM monitoring but in most of the organisations EM will be done by Microbiologist only. It's depends on Analyst Qualification /procedures adapted/ Risk assessments.

Most of the batch manufacturing records have the EM is performing / not, but they won't write it's results. This practice is like common area state of controls like DP, Temperature, RH etc...

If any executions observed / notified, then Manufacturing team will do repeated cleaning and perform investigation as part of OOS.

A4: In some companies, Ops performs EM because they are in the area at the time of the activities. QC has oversight if that occurs.

A5: Recording of EM monitoring in production log book is of course mandatory because as performing monitoring is similar to usage of Equipment like LAFU,DBP etc hence you have to record the data, of course data is redundancy as same replicated in qc books. You can minimise thorough a justification report .

A6: 20 years ago I started my career auditing manufacturing folk take samples because FDA observed manufacturing folk ….. simply not taking the samples carried into the cleanroom with them. It’s a huge conflict of interest issue but yes, they would need to be watched by someone from Quality in order for this to be deemed acceptable.

A7: If documentation in microbiological laboratory complies as per data integrity policy.. I would suggest not to make entry I production area.

A8: Ask production or who prepares the respective SOP to define the scope of the production log book. Usually, companies have a log book to capture all the activities being done in each manufacturing room, in this case, EM also is an activity which need to be recorded, unless it is specified in SOP that this production log book is for production activities and for Microbial EM refer the respective EM log book.

A9: You are recording the details of EM in your log book or EM record, whereas production unit have to mention each activity performed in manufacturing areas. So if they are mentioning EM activity in their logbook. It is absolutely fine and advisable so as to doubly verify the EM controls present in your organization.

A10: If you're including finger / sleeve plating for bias sterility samples as "EM" here, that should go into the applicable batch record.

Method Verification for an Antibiotic Ointment assay for the active

What are the parameters that need to be taking under consideration in performing a method verification for an antibiotic Assay.

A1: Please consider following

Precision
Accuracy (recovery)
Specificity
Ruggedness
Robustness

Linearity you already perform for the five level standard preparation.

Raw materials limit establishment

I need some clarity regarding raw material limits establishment.

Vendor specified total 10 microbiology test parameters for Fructo-oligosaccharides raw material.

This raw material used for vaginal products, based on the end product, I applied vaginal category test parameters for raw material as per USP 1111.

Can I follow this concept or not?

A1: The raw material is an end product for the API vendor hence they perform the multiple parameters to meet the requirements of all the market and customers . As an end user you will verify material intended purpose i.e as per the requirement of USP <1111> .Inhouse Rationale or justification document shall be maintained to justify the regulatory audits.

A2: I suggest to adhere USP 1111 but however it's not mandatory chapter

So I would prefer to perform Risk based approach w.r.t to additional pathogens which is not part of USP 1111.

water system for steam qualification

A friend is installing a small pure water system for their steam generator. The steam is used in an autoclave for final product sterilisation. No product contact. Is a performance qualification of this system of 5 days followed by monthly testing sufficient?

A1: I assume that none of the guidelines describes the frequency of pure steam generator qualification ,it depends on firm to set the frequency and generate data. Once you get enough data you can set the frequency by considering impacting factors.

Sterility test- particles

In our laboratory sterility testing is performed in a Class A laminar flow. This is monitored every session for particles.

An auditor claimed that it is standard practise to monitor the surrounding areas for particles.

This includes the work room and pass through - Class B. Gowning room and corridor - Class B/C.

How often do people monitor the Class B and C areas in a sterility testing suite?

Would once every three months be sufficient?

Is it standard practice to monitor sterility suites for particles?

A1: If you use ISO 14644 and USP 1116 as a guide, your Grade B areas should be sampled with each sterility session/shift and your Grade C spaces should be sampled every day. Here are two additional references:

“The working conditions in which the tests are performed are monitored regularly by appropriate sampling of the work area and by carrying out appropriate controls“ (EP, 2.6.1/ USP, <71>)

“Environmental monitoring should be performed under operational (dynamic) conditions either within the isolator or in the laminar airflow and associated background areas” (PIC/S 2007)

In general, because you're operating in a hood and not an isolator, I'd treat it as a higher risk and have a sampling plan for your entire suite.

A2: There is no need to monitor for particulates during sterility testing. Who was this auditor?

A3: The sterility test bench/hood is essentially a Grade A environment, and should be monitored continuously during sterility testing performed within its space. This ensures that the hood is as clean, ideally cleaner than the filling zone in which product was filled/compounded, etc. The rationale is that any testing performed here reflects directly on the filling environment - not this local one, as any 'fail' result here will impact production, regardless of where the actual contamination event occurred.
As such it is also common practice to ensure that the surrounding area is also free from contamination , and monitored regularly to ensure that contamination ingress does not occur.

10. It is recommended that a similar system be used for Grade B zones although the sample frequency may be decreased. The importance of the particle monitoring system should be determined by the effectiveness of the segregation between the adjacent Grade A and B zones. The Grade B zone should be monitored at such a frequency and with suitable sample size that changes in levels of contamination and any system deterioration would be captured and alarms triggered if alert limits are exceeded. - EU Annex 1 - 2009

Monitoring each shift within the Grade B, during which testing in the hood is performed can be done easily using a portable instrument, that can then be connected to a hood mounted probe for sampling during

testing within the hood space. A second test on completion within the Grade B would demonstrate that conditions have been maintained. If the suite is not used every day then this would also be suitable, for each test period.

B/C corridors and change rooms, each week.

A4: I would not assume that regular means daily particle monitoring. Periodic monitoring may have been a better choice of words.

Particulate matter test _ for coloured injection

We have a coloured injection dark blue in colour, and we want to analysed for sub-visible particulate matter test. Any one has an idea how to analyse it?

A1: Have you tried a light obscuration particle counter instrument? Although the dark blue colour may appear near opaque in a beaker, the capillary within the instrument is only a few hundred microns wide and often will transmit sufficient light to observe obscuration events. It can be verified by spiking a test sample with particles from a known size standard and ensuring they are present in the correct size. Failing that it is either dilution or microscope.

A2: I would refer you to USP <788> Particulate matter in injections.

Environmental Monitoring of Viruses and Mycoplasma

Is there guidance on EM for viruses and mycoplasma in cleanrooms? Can someone point me in the right direction?

A1: There is not. There is no practical method for conducting EM sampling for viruses or mycoplasma. What leads you to believe this is necessary?

A2: Viruses and mycoplasma of clinical significance usually do not survive outside the human body and are readily destroyed by disinfectants used in cleanrooms.

Absence of guidance documents for viral environmental monitoring should be viewed as indicative that it is not necessary.

The issue is briefly discussed in the article I co-authored: Cundell, A.M., D. Guilfoyle, T. R. Kreil and A. Sawant 2020 Controls To Minimize Disruption of the Pharmaceutical Supply Chain During the Covid-19 Pandemic PDA J. Pharm. Sci. & Technol. May 2020, pdajpst.2020.012021; DOI: https://doi.org/10.5731/pdajpst.2020.012021

A3: Bad move. Sounds like a "bug hunt" intended to find microorganisms that may have an impact on product quality. There is no such guidance that you're looking for. Dr. Sutton eloquently described the purpose of an EM program several years ago:

“The purpose of the EM program is to document the state of control of the facility, not to determine the quality of the finished product.”

Sutton S. The Environmental Monitoring Program in a GMP Environment. J GXP Compliance 2010:14(3)23-30

Sodium thiosulfate used in city water testing

I am interested in the way that people are using/making sodium thiosulfate for the testing of city water that contains chlorine. I have previously seen sterile cups which contain the sodium thiosulfate, and I believe came from an outside vendor. Can anyone comment on how they are performing their sample prep for chlorinated water? Either the vendor that they use to buy pre made containers, or how they make their own sampling system (along with the concentration of the sodium Thiosulfate used?

A1: We use a commercially available sterile cup that includes a sodium thiosulfate tablet. The cups are graduated to show the target fill volume to ensure the correct concentration of sodium thiosulfate is obtained. I will refrain from mentioning a specific manufacturer since some of the sponsors of this forum may have their own version of this product.

A2: You can use 0.1 mL of 10% w/v sodium thiosulfate in a container and sterilize it. Moreover you should, validate the actual quantity of 10% sodium thiosulfate required to neutralize the chlorine present in water, based on outcome you decide required quantity.

Do you need to replace Nitrogen with Compressed Air

Our product (vial fill) has a nitrogen overlay. Does anyone know if there is a requirement to replace the nitrogen overlay with compressed air during a media fill? Otherwise, is it sufficient to perform all necessary interventions in setting up the nitrogen overlay including having each vial stop under the nitrogen needles but just with the nitrogen turned off?

Note: We understand compressed air replacement for nitrogen is required for a lyophilization vacuum break.

A1: Hi Dina. This is from PIC/s: 4.1.3 It should be considered that inert gases will prevent the growth of aerobic microorganisms. Therefore for process simulations sterile filtered air should be used instead of inert gases, also for breaking a vacuum. Where anaerobes are detected in the environmental monitoring or sterility testing, the use of an inert gas should be considered for a process simulation, as inert gas is supporting the growth of anaerobes.

Normalizing Non-Viable Particulate Count Values

As I continue to mature in my nascent GMP career, I am seeing more and more companies that attempt to normalize non-viable particulate counts obtained during dynamic conditions. For example, I am currently working for an employer that samples one cubic foot of air during dynamic conditions, and applying a conversion factor of 35.3 (1 cubic meter = 35.3 cubic foot) in order to normalize the raw data captured to 1 cubic meter.

I was under the understanding that this practice is highly discouraged for a multi-product facility that depend on aseptic processing to deliver “sterile products,” owing to (among other things),

a. variability in the manufacturing steps required to ensure CQA (critical quality attributes) for each product, and
b. variability in CPP (critical process parameter) for each product.

I could see how normalization may be justified should a company produce one single product under closed environment (e.g. closed isolator) after evaluating historical trends during dynamic conditions, but I simply do not see how the aforementioned normalization can be justified for a multi product company that depend on aseptic processing to manufacture sterile products.

Please advise if I am mistaken and educate me as to other instances where normalization of non viable particulate count values can be justified.

A1: If the air cleanliness is expressed in counts per cubic meter then you must collect a 1 cubic meter sample.

A2: The sample from a particle counter is a function of observed raw counts within a fixed interval of time, and is used to demonstrate control over a process. Normalization is singularly an effect of putting said data into a format correlative to a standard, not necessarily reflective of control itself, although parallel to. Environmental control should be a function of data/time regardless of units, the same as looking at differential pressure in mbar. The act of normalization itself is not in error, but what is done with the data showing an observed change in conditions, requires more consideration.

TSA plates at two temperatures

In Class A and B rooms. If environmental monitoring is done on TSA plates with incubation at two temperatures, 30-35C then 20-25C, is it necessary to read the plates before transfer to the next temperature or just to read at the end of total incubation time?

A1: I'm not sure that there is a solid regulation that requires intermediate reads, however catching an alert or action that's been exceeded early gives you a head start on investigating a root cause.

A2: No there's no "requirement" to read the plates but some firms do an interim read.

A3: In Invert position Petri dishes are incubate at 30°C -35°C for 48 to 72 hours for bacteria and For or Total Yeast and Mould counts incubate at 20-25°C for next 5-7 days.

After the incubation, examine the plates for growth, count the number of colonies and express the number of microorganisms in CFU.

A4: Hilt is must to read the colonies before transferring the plate to next temperature.

A5: In regards to this posting,I have no objections that an interim microbial count be performed between the two incubation temperatures. However, I do have a question concerning the order of incubation temperatures by using a single TSA Petri dish that people had indicated. I was always under the impression that the TSA plate be first incubated at 20-25C for 3-days and then at 30-35C for 48 hours. It was my understanding that this order of incubation temperatures was to allow yeast and mold to grow first to be detected and then incubated at 30-35C to detect the presence of bacteria since they would not die-off at 20-25C. If you allow a bacterial spreader to grow first by incubating at 30-35C, you might not be able to detect the presence of yeast and mold in air. Does my logic make sense concerning the order of incubation temperatures?

A6: Read after the incubation, intermediate reading is not required .Multiple readings leading the errors.

A7: It's a catch 22. Dual temps will always favor fungi over bacteria or vice versa. If you go low to high, molds may mask bacteria. High to low, bacterial spreaders can mask fungi. It's a good happy hour discussion.

Ideally, you can pick any temperature scheme that demonstrates the highest recovery in YOUR facility. Plenty of studies have demonstrated that dual temps are less favorable that single range.

There is no definitive answer that can be offered for incubation scheme, it's trial and error and some validation mixed in for luck.

As far as interim reads. Do it. Might as well sneak a peak before any organisms decides to ruin your life and stretch out over the plate. It's not required at all (neither is any specific incubation temp either!), but, it's a good practice.

Just my $0.02.

A8: At end of incubation

A9: I agree, that is what the sequence should be. Incubating at greater than 30C first, will deter Yeast mold growth

A10: Based on our experience with fungal infection including the NECC outbreak, pathogenicity is related to the ability to grow at 37 degree C so higher incubation temperature greater than 2-25 degree C may be preferred with a risk-based approach.

All microorganisms have an optimum, minimum and maximum growth temperatures. A single temperature around 28 degree C may be the best approach.

A11: We can include the "just 1 temperature incubation" for recovery of both fungi and bacteria on that happy hour talk to make it more interesting.

A12: I am glad you raised this single temperature setting concept where no need to shift plates from lower temp to higher. In my experience, a 30C +/-2 degree set up satisfies for most environmental isolates bacteria and yeast/mold.

If one is specifically looking for thermophilic organisms, a higher than 35C temp. requirement, a separate set of plates can be set up.

A13: Several of my high school lab folks ran studies with different strains of fungi and wild strains and found faster growth for all but one strain at 37 deg C. Since people have so many fungal infections, it always surprises me when people think that molds won't grow at 35 deg C.

A14: Why not use two plates incubated in parallel at respective temperatures? Provides more timely completion of assessment and removes potential confounding factor of serial incubation.

A15: Even at 30C +/- 2 incubations, we do plate them separately on TSA and PDA/ MEA plates. I understand that many QC labs prefer to minimize plate usage and prefer t single plates but incubate long days.

A16: I can’t say I’m a fan of single temps, but, to each there own.

I try and remind people that were aren’t looking to recover 100% of our cleanroom organisms. We want enough to be able to make informed decisions, find root causes and implement process improvements.

A17: The background. Sterile gloves are monitored on TSA only. Presently at 30-35C. An auditor requested that we do two temperatures.

A18: Do you have a problem with yeast and mold? Have you shown you can recover yeast and mold at one temperature? What was the auditor's justification considering there is no consensus in the industry and there is data showing all three incubation scenarios can work? It would make more sense if they asked to see the data showing your recoveries of YM at one temp, but to dictate a new incubation scheme, that seems premature.

A19: By auditor do you mean internal auditor, client auditor, or regulatory agency auditor.

I agree with [name redacted] this is a monitoring program not an absolute count design to detect a loss of environmental control.

Find a polite way of telling the auditor to get lost.

A20: if an auditor specifically is requesting two temps, he or she may have observed or perceived conditions in your facility such that they believed there was reasonable risk of operators tracking around

organisms that are associated with general “filth” such as spore formers, gram negs, fungi, as well as risk of operators breaching their own gowns with the glove, or the glove otherwise becoming contaminated with human borne flora. Look at the request in light of other observations made, and it might make more sense.

A21: I’ve been following this thread of emails with interest and, with all due respect, would like to put my 2 cents in! More than one client of mine over the years has had FDA auditors request/demand that the two incubation temperatures be used whether with one media or two so that the media/incubation scheme will allow for good recoveries, especially for mold.

One client had qualified a single (monophasic) incubation scheme at 290C with TSA/ TSA LP80 plates for 5 days. Although it was several years old, it had been well thought out with a variety of EM isolates, including molds. The FDA auditor informed the client that “everyone uses biphasic incubation” and the FDA auditor would not back down, even when told diplomatically to get lost. So I worked with the client to put together a good, robust study to evaluate the best media/incubation scheme for their site that included:

* 5 days at 290C
* Single TSA/TSA LP80 plates at 20-250C for 3-5 days, followed by 30-350C for 2-3 days
* Single TSA/TSA LP80 plates at 30-350C for 2-3 days, followed by 20-250C for 3-5 days
* TSA/TSA LP80 plates at 30-350C for 2-3 days in parallel with SDA/SDA LP80 plates at 20-250C for 3-5 days

We used EM isolates that included Gram positive cocci, Gram positive non-spore-forming rods, Gram positive spore-forming rods, yeast, Gram negative pseudomonad type rods typically recovered from purified water systems, and several molds, including an ATCC Mucor that would only germinate at <250C. Only one isolate was plated per plate.

Plates were checked daily to observe progress and identify any fast-growing mold or spreading bacterial colonies.

The Mucor species would not germinate at 290C, 30-350C or the scheme that started with 30-350C. Mucor, once germinated went crazy within 48 hours and would cover the plate. By far the best scheme for single TSA/TSA LP80 plates was 20-250C for 3-5 days, followed by 30-350C for 2-3 days. Aspergillus and Penicillium isolates “preferred” this scheme even over SDA/SDA LP80 at 20-250C for 3-5 days.

Realizing that other sites may not have the same results, the key point is that the site should do a suitability/qualification study similar to the one above to determine the best media/incubation scheme for their site location. Different climate zones may have different results. This study design provides data that can justify the media/incubation scheme selected to hopefully appease our friends at the FDA, which this client site was able to do.

A22: My only comment here is that no FDA inspector should “demand/require” any incubation scheme. If you refer to the 2004 aseptic guidance, they recommend high temp for bacterial counts and lower for yeast and mold. This is not an indication nor requirement to utilize dual temps or any combination thereof.

Had this been included on a 483, I would have respectfully challenged that observation with my rationale and supporting evidence supporting my EM incubation conditions and trend data. I would even consider an arm wrestling challenge!

No regulatory agency should tell a firm what their EM conditions should be. A firm should be able to have scientific rationale behind their incubation conditions with validation data to support their scheme. This would then be defendable to an agency representative.

Again, there is no “pre-set” scheme that universally works. Much of it is science with a hint of trial and error and a pinch of luck.

Don’t forget Murphy’s law of microbiology:

Despite the most favorable conditions of time, temperature and incubation, microorganisms will do whatever they damn well please. Especially, when your job depends on it.

A23: While I agree with your thoughts, the reality is that it was a 483 observation and the client site did challenge but still had to do the study. If the single temp/single media incubation scheme had shown the best response, I'm sure the client site would have pushed the issue. As it was, the data showed the single media/dual temp incubation scheme that started at 20-25C had the best recovery.

Recovering bacteria and molds from the cleanroom environment is never black and white but you do the best you can. It's all about trends anyway, not a single result. One final note: The client site switched to the qualified media/incubation scheme and a higher percentage of their recoveries were mold. So, coincidence or was the previous scheme not adequate to recover the mold that hit the plate? Maybe it was Murphy's law or maybe just the right site was sampled at the right time? Or maybe the incubation scheme that was implemented was an improvement?

This is what makes our job interesting and often times, fun.

A24: I would like to introduce more aspects to discuss in this interesting conversation.

Have companies that perform a non-standard incubation process for environmental monitoring samples (1 incubation at a different temperature than indicated in USP1116) adapted the Growth Promotion Test (GPT) process of the medium or do they still maintain the regulatory strategy established in USP61?

What incubation strategy do you perform when doing the aseptic fill test?

A25: Are you asking if you use alternative incubation strategies as outlined in USP 61?

A26: Right [name redacted]. All one has to do is point to the 2004 FDA Aseptic Guidance document. That being said, if a single temp/medium is used, I would expect the firm to have performed a validation to demonstrate that those incubation parameters/media were optimal at recovering microorganisms.

A27: This is a long thread.

USP <61> is for microbial enumeration for non-sterile drug products so non-compendial environmental monitoring is not strictly an alternative method to a compendial test as designed in USP <1223>.

The media selection and incubation conditions should be viewed as a compromise suitable for detecting a loss of environmental control in your manufacturing facilities.

With numerous studies published in the peer-reviewed literature and book chapter I believe companies should make reasonable method selection and forgo so-called validation studies. With closed systems becoming the state of the industry, it is time to back-off excessive environmental monitoring. For example, why conduct routine environmental monitoring in a gloveless isolator.

A28: Of course it is necessary to read the plates before transfer to further temp. Incubation, general practice is first incubation at 20-25 dgr temp.so you can read the plates for fungal or mold count comfortably, then incubate at 30to 35 dgr temp.for bacterial growth.

A29: I agree. Process controls should supersede redundant EM, however as in all scientific endeavors, it takes time to make changes.

Regarding the temperature controversy, I recently had a client in which this conversation became quite contentious. The bottom line was that there are data sets that differ in their conclusions. For example: https://pubmed.ncbi.nlm.nih.gov/25336415/

The following statement appears in this article:
We found the highest recovery of total aerobic count from areas with personnel flow using a general microbiological growth medium incubated at 30-35 °C. The highest recovery of moulds was obtained with mycological medium incubated at 20-25 °C. Single-plate strategies (two-temperature incubation or an intermediate incubation temperature of 25-30 °C) also yielded reasonable recovery of total aerobic count and moulds. However, recovery of moulds was found to be highly inefficient at 30-35 °C compared to lower incubation temperatures. This deficiency could not be rectified by subsequent incubation at 20-25 °C.

A second source is from Dr. Tim Sandle:
https://ijpc.com/Abstracts/Abstract.cfm?ABS=3832

Abstract: A study was undertaken to compare microbial recoveries from pharmaceutical grade cleanrooms using two different incubation regimes and a general-purpose agar (Tryptone Soy Agar). One temperature regime (A) incubated plates first at 30°C to 35°C, followed by 20°C to 25°C; the second temperature regime (B) began the incubation with plates at 20°C to 25°C, followed by 30°C to 35°C. The experimental outcomes demonstrated that there was no significant difference with the total microbial count when measured using a t-test (0.05 significance level; 95% confidence interval). However, with the recovery of fungi, the second incubation regime (B), which began with the lower 20°C to 25°C temperature, produced higher incidents and numbers of fungi. While this finding might provide the basis for adopting one incubation regime over another, a review of the types of cleanrooms recovering fungi suggests that fungal incidents are low, and they are more often confined to specific areas. Thus, as an alternative, incubation regimes could be varied to suit different cleanroom environments or a selective mycological agar adopted for specific areas.

The third citation is from USP <1116>:
Selection of Culture ConditionsTime and incubation temperatures are set once the appropriate media have been selected. Typically, for general microbiological growth media such as SCDM, incubation temperatures in the ranges of approximately 20°–35° have been used with an incubation time of not less than 72 hours. Longer incubation times may be considered when contaminants are known to be slow growing. The temperature ranges given above are by no means absolute. Mesophilic bacteria and mold common to the typical facility environment are generally capable of growing over a wide range of temperatures. For many mesophilic organisms, recovery is possible over a range of approximately 20°. In the absence of confirmatory evidence, microbiologists may incubate a single plate at both a low and a higher temperature. Incubating at the lower temperature first may compromise the recovery of Gram-positive cocci that are important because they are often associated with humans.
The bottom line is that incubating at lower temperatures followed by higher temperatures will support the growth of fungi and bacteria, respectively, with the caveat that Staph and other gram positive cocci may not be effectively recovered.
Thus the jury is out and hopefully these references can be used to support your incubation scheme. Or to cloud the issue.

A30: With many companies incubating and reading around 20-30 or more thousands of samples per month, you are saying just double the amount of reading by doing interim read? There is absolutely no requirement, need or value in doing interim reads, you are looking in the past anyway. Having info few days before won't make significant changes in a robust QC system.

And I didn't even mention resources to do such a thing (people, money, time, repetitive strain issue).

A31: Don’t forget the PDA Journal paper from GSK folks a few years back that indicates dual temp provided the lowest recovery.

To each their own! "Validation" of EM incubation scheme does not need to be a lifelong endeavor. A small-scale, in-situ study can be performed.

As many of us have stated, spend more time preventing contamination than hunting it down.

Viscosity drop in stability due to cellulase

I have observed in some of the products having Hydroxyethyl cellulose that viscosity is decreasing after few months.

I have researched and found that though microorganisms are killed - cellulase enzyme remains in the formulation as a result viscosity drop is observed.

I was wondering if there is any method available to quantify the presence of the 'cellulase' enzyme.

The product is non-sterile cosmetic grade.

A1: Cellulase-containing microorganisms are relative rare and are unlikely to be in hydroxyethyl cellulose given its manufacturing process.

What is the pH of the solution? At low pH aqueous solutions <6.0 may undergo acid hydrolysis with a resulting lower viscosities. Not everything bad is caused by microorganisms.

A2: You need a fermentation level load of microorganisms to produce so much cellulase that it will break down cellulose in your product. Did you have such a high load to begin with?

If not, I suggest you look at the buffering of your formula. Shift in pH might lead to viscosity changes.

Use of Burkholderia cepacia as a AET Organism

I will admit that I do believe in using *Burkholderia cepacia *as a challenge test organism to determine the preservative adequacy of aqueous product formulations for the development of very robust preservative systems against the introduction of microbial contamination during consumer usage.

I have been hearing from other consultants and contract testing companies that the agency has been asking companies to include *Burkholderia cepacia *as one of the AET test organisms in their NDA and ANDA submissions. In addition, I have also heard that the agency is asking some of the generic manufacturers to add *Burkholderia cepacia* as part of their approval process. I do not have any objections to this type of request from an agency

When an agency makes this type of request for a company, are they asking just for the inclusion of* Burkholderia* *cepacia* (e.g., ATCC 25416) and not including *Burkholderia cenocepacia *ATCC BAA-245 and *Burkholderia *m *ultivorans* ATCC BAA-247 as a challenge test organism? Just wondering. I'm sure that when the agency is making this request that it is not as a suggestion but as a demand from Mt. Olympus.

I'm sure that most companies are afraid to ask why the agency is making such a request for the inclusion of *Burkholderia cepacia *as a challenge test organism.

I do admit that *Burkholderia cepacia* complex contamination in non-sterile products is a very large issue for the industry because of poorly designed purified water systems and errors that occur during cleaning and sanitization of manufacturing equipment. From my understanding, you do not use preservative systems to correct manufacturing issues. I believe that I saw this once in either a 483 citation or a warning letter. Is the agency making this request for including *Burkholderia cepacia *as a challenge test organism to take care of manufacturing issues?

In the past, I have conducted microbial in-use studies in which products were used by consumers and the microbial content of these used non-sterile product formulations was determined afterwards which is way

different from conducting microbial challenge testing. This type of microbial in-use testing is more commonly performed by cosmetic companies than drug manufacturers. From what had been obtained, I have found that the microbial bioburden of these used contaminated products mostly consisted of *Staphylococcus *species (majority was *S. epidermidis*), yeasts and Gram-positive bacilli. Occasionally, we were able to isolate coliform bacteria from a used product formulation and found out that the user was using well water instead of city water as their potable water supply. We were also able to detect *S. aureus* carriers by performing this type of testing. HR and legal got their noses out of joint over this. In my experience, I have never been able to isolate *Burkholderia cepacia *complex from a used product formulation by a consumer. The reason may be that I had a very robust preservative system in the formulation in place against *Burkholderia cepacia* complex. Have other people been able to isolate *Burkholderia cepacia* complex after a consumer had used a product that had initially been found to be free of *Burkholderia cepacia* complex after conducting Quality Control release testing?

A1: My two cents - I recently was involved in a nonsterile plant agency audit, and BCC was at the forefront of the inspectors mind (caveat - inspector was a chemist….). The site outsourced their AET, but as mentioned in a previous thread a few weeks ago, they were all over the fact that positive ATP sample results (Celsius) were only streaked on TSA and not BCSA. If I were to guess, if this particular inspector was asked if Bcc (and yes, I'd think the three Burkholderia species would be expected) should be used in AET testing, the answer would have been yes, judging on the rather intense focus on this complex of organisms.

A2: This is an interesting point, considering the Celsis system cannot be used to conduct an AET (USP 51) test, unless it was being used as a screen. The Celsis system is a qualitative ATP method and cannot provide an absolute cell count that can be compared with the USP 51 acceptance criteria.

A3: I too support [name redacted] suggestion. Esp. for cosmetics, I see it as addressing capability of microbial adaptation (to preservatives) not found with the classic challenge bugs. https://academic.oup.com/femsle/article/144/2-3/117/639711?login=true

A4: It would be a better approach to streak out on a non-selective medium like TSA and characterize the colonies to screen for objectionable microorganisms than going directly to BCSA. For example, if P. aeruginosa was in the enrichment it would not grow on BCSA.

You must make the case to a non-specialist to justify your policies and procedures. I would refer you both to the 2014 Technical Report No. 67 *Exclusion of Objectionable Microorganisms from Non-sterile Pharmaceutical and OTC Drug Products, Medical Devices and Cosmetics*

A5: That is correct [name redacted]l. To clarify, in the case I presented, the Celsis was used as a screen, and if the screen turned up positive, it would be struck on TSA to obtain a count. Inspector seemed to want to see it struck on BCSA also at that step. Not a bad idea in my opinion. This was for basic limits testing, not AET.

A6: Then in this case, it can be inoculated into TSB and after an incubation period, transferred to BCSA, similar to the directions in USP 60. This may provide a more robust assay for the detection of BCC.

A7: When you streak onto a selective/differential agar, you will get a presumptive identification of the recovered isolate if it shows characteristic biochemical reactions, but you will still need to subculture onto TSA for Gram-staining and biochemical identification. So why use a selective/differential agar to confirm a ATP positive test result? It just adds complexity in confirming a positive ATP test result.

As far as using a ATP bioluminescence test as a screening tool for preservative challenge testIng, I would advise against it because as Michael had stated that it is not quantitative unless you run a MPN scheme for each challenge test organism. Furthermore, not all preservatives that are used in product formulations have cidical activity to reduce the all of counts for each challenge test organism to zero. Many preservatives are just inhibitory to prevent the grow of organisms especially against fungi and they do not kill all of the challenge test organisms.

In the end, are regulatory agencies asking the inclusion of B. cenocepacia and B. multivorans in addition to B. cepacia when they requesting that B. cepacia be used in performing AET on a product formulation? It is my opinion that the usage of only B. cepacia should be more than sufficient for conducting AET for their request because you cannot perform challenge testing on a formulation by using each microbial species that is known to man.

A8: I hear what you're saying [names redacted]. How this particular lab's system was set up was a bit unconventional, on top of the whole "failed to ever qualify media per <61>" issue which in my opinion invalidates almost every test coming out of there in the past decade or more. This lab was using the Celsius as a screening tool. If no ATP, the test ended there. If there was a positive, an enumeration method would then occur. I think the inspector wanted to see BCSA because the assumption is made that TSA alone doesn't give an accurate count, if we are concerned with Bcc being included in that count. Because TSA doesn't detect all subtypes, etc. But the issues were much larger. I had to strategically duck out of there, and now they have a master chemist, that knows enough micro to make him dangerous, fixing their micro methods. These particular products have antimicrobial properties with preservative systems. PET testing is outsourced.

A9: ATP screening, <60> and <62> are limit tests not enumeration that give a result of absence in 1 g or mL.

With the ATP screen you can qualify the enrichment and isolation of Bcc and adjust the enrichment broth and perhaps agar to half strength and increase incubation time.

A10: I won't discuss AET or the need to properly validate the Celsis as an alternative method. But I will point out that using TSA to recover a total aerobic microbial count in a USP 61 test is in compliance with the compendium. Any inspector that thinks otherwise should be strongly educated to this fact, and if a company stills gets a 483 then this should be the response to FDA. Finally, I will remind the group that In 2004, GSK validated a qualitative ATP method for a nonsterile nasal spray in which any positive reverted back to the compendial test. But this was thoroughly validated to be equivalent to the compendia test. They obtained the very first FDA PAT approval for this application. Michael

A11: If the laboratory was not performing growth promotion on their microbial media, I would not be surprised that they were not also performing validation of the ATP Bioluminescence test method or their traditional enumeration and enrichment test methods. Because of personal experience, I will admit that I have never been a big fan of chemists or toxicologists being responsible for running a Quality Control microbiology laboratory, but I do believe that there are some chemists in the industry that are able to run a microbiology laboratory because I have met some of them. I have found that these individuals have taken many microbiology workshops on microbial content testing, preservative challenge testing, sterility testing and endotoxin testing to increase their knowledge.

As far as using BCSA as a plate count agar, I'm surprised that the inspector had made that recommendation. It shows that the inspector has very limited knowledge of microbiology. I have always question the usage of selective/differential agars for enumerating the presence of certain types of microorganisms such as using MacConkey Agar for enumerating Enterobacteriaceae and Cetrimide Agar for enumerating Pseudomonas aeruginosa in a test sample. I was trained to use selective/differential agars as a presumptive presence or absence test for certain types of microbial species in an enrichment test sample and not for enumeration.

I can see using BCSA if you want a presumptive identification of the recovered contaminant in a positive ATP Bioluminescence test result, but it does not add anything more than that just using TSA as a recovery agar for Gram-staining and identification. Furthermore, I have never heard of TSA not being able to isolate, grow and enumerate Burkholderia cepacia complex from a test sample. I wonder where the inspector got that idea, The purpose of enrichment is to increase the levels of organisms in a test sample. I would be more concerned about other organisms preventing the isolation of Burkholderia cepacia complex in an enrichment test sample by the completion of nutrients. In my experience, I tend to see Burkholderia cepacia complex as a single contaminant in an enrichment test sample and not as a mixture of microbial contaminants. I do know that Burkholderia cenocepacia and Burkholderia multivorans are slow growers in

comparison to Burkholderia cepacia and that is the reason that the incubation time is 48 to 72 hours in USP Chapter 60 for the incubation of TSB and BCSA. Because B. cepacia, B. cenocepacia and B. multivorans are recommended for usage in USP Chapter 60, I always make the recommendation that they be included for validating ATP Bioluminescence or any other rapid microbial test method of aqueous product formulations.

A12: Thank you for that clarification and ideas [name redacted].

From this, I believe what I had recently seen was clearly misusing the intent of this test. I believe enrichment (for Celsis testing) in (improperly qualified) TSB was 24 hours. If no ATP activity was seen, the sample passed.

Out of curiosity, if the enrichment was being qualified for Bcc, would the three common Burkholderia species be used here? I see the logic in adjusting the broth to half strength, but if incubation time is increased, wouldn't this be moving in the other direction from 'worst case'?

A13: Excellent points [name redacted], and thank you. I think Chemists absolutely could pick up on the subject matter academically, and learn some great info in seminars, etc. But if they need to train a lab of microbiologists, it might be a train wreck seeing what type of aseptic technique is transferred to the lab. That takes years of practice to fully grasp. I had the fortunate opportunity of supporting my college microbiology lab for four years, and came into industry with that mindset. It took time above that working in a hood to really get the technique(s) down. I can't personally see anyone jumping into that adequately without the time / experience.

Growth Promotion of MRSA

What would you recommend for doing GPT on Lactobacillus MRS agar? Is there any reference out there?

A1: I do not know of a particular reference, but I do expect that you should be performing growth promotion testing of Lactobacillus MRS Agar if you are making a dietary supplement such as a probiotic containing Lactobacillus species to confirm the number of organisms present in the product. It is good cGLP practice to conduct growth promotion testing of all laboratory prepared microbial growth media to ensure that a lot can support the growth of microorganisms when used in testing.

As far as the growth promotion organisms, each manufacturer of Lactobacillus MRS Agar has their own specific growth promotion test organisms. Whatever brand you are using, I would recommend that you use the listed organisms that the manufacturer uses. In addition, I would also use the strains that are present in the product as growth promotion test organisms.

A2: We use these organisms prior to release. I'm sure a subset would work.

Lactobacillus acidophilus ATCC® 4356
Lactobacillus sakei ATCC® 15521
Lactococcus lactis spp. Lactis ATCC® 19435
Pediococcus pentosaceus ATCC® 33316
Pediococcus pentosaceus ATCC® 29358

Culture media for Algae

Any culture media/method/reference for detection of algae you can recommend?

A1: Algae!

When you ask detection of algae, what are you referring.

Can you be little more specific on detecting algae from what system/location, substrate, environment etc.

As you may know there are many kinds of algae, one requiring no/ nitrogen (nitrogen fixing algae), there are green algae which needs nitrogen.

Some basic universal algae media are Allen's Medium, Bold's Basal Medium, BG11 medium etc,. If you just search for these media, you will find informations on web.

You will also need an incubator set to light and dark cycles.

A2: Dr. Sadasivan - If it's photosynthetic and microscopic, she knows about it!

Stability

An accelerated stability program was set up at 40C for an injectable . It included upright and inverted vials. No closure integrity is performed. Why should there be sterility testing separately for upright and inverted vials?

A1: A better question would be why would sterility testing be a stability test parameter under accelerated storage conditions?

A2: I agree with [name redacted] comment about testing under accelerated storage conditions.

On the topic of why test both upright and inverted orientations as part of stability, that is done to determine the effects and possible interactions of the drug product with the closure. You can find this information in ICH stability guidance.

Expiry of sterile product

We have a gamma sterilised packing material, whose expiry was June 2021.

We want to use it in July 2021, what should we do? What documents shall we generate or get from the Manufacturer?

A1: It is not justifiable to use after expiry, as it validated process by manufacturer. Incase if you use on risk based ,have to justify the results don't where how it would be either of it. My suggestion is better don't deviate the assured expiry date usage.

A2: To use a product after expiry is never an acceptable practice. What you could do is ask the manufacturer if they have any studies on multiple ionizing radiation treatments. If multiple radiation treatments do not impact the quality of PM then; I highly recommend you send material to get sterilized by gamma and use it. As packaging material is in direct contact with the product best way forward is to consult with the regulatory department.

A3: We are planning to get it ETO sterilized, after the treatment which tests are recommended besides sterility test.

Conductivity testing of purified water/WFI

When calibrating your conductivity meter what standard do you use to perform the calibration?

We are looking for limit of 1.3us/cm (<USP>).

We used to use a 10us/cm standard but switched quite a few years ago to use a standard closer to the test value limit as i think it was stated within the USP chapter that it needed to be similar.
We now use 1.3us/cm standard and calibrate the meter before each testing session.
The standard that we purchase is £130 per bottle and it expires on the day of opening causing us a significant cost for testing conductivity. The 10us/cm standard was significantly cheaper and i think had a 3 month open expiry date.

A1: I can say with high confidence there is no reliable (within 2%) reference conductivity solution available at <10 uS/cm, not one that can withstand reasonable shelf life tests, or have a more-than-1-time use. This is because the infusion of air, CO2 specifically, will impact and increase the conductivity. Likewise, the ionic content of a 1.3 uS/cm is so low that any impurities on the sensor or in the measuring container will alter the conductivity. That is why these solutions are not readily available.

But the greater misconception is that one "must calibrate the sensor in the range of operation". This is true of pH, this is true of a balance, and this is true for many other analytical methods. But for conductivity, the sensor is calibrated by determining the cell constant (cc), and the cc is a physical and geometrical property of the sensor, and it has units (cm-1). As long as you are measuring/calibrating in the operating range of the system, you are free (and it is proper) to calibrate away from the normal operating range. Calibration in a ~100 uS/cm is perfectly fine, even when it is normally measuring 0.05 to 2 uS/cm, for example, and no reference solution exists.

An alternative approach is to obtain a reference cond sensor, and do a side-by-side calibration in the same container of water, or inside the water system.

See USP <644> Conductivity of Solutions and USP <1644> THEORY AND PRACTICE OF ELECTRICAL CONDUCTIVITY MEASUREMENTS OF SOLUTIONS for the justification of these approaches. They are not new or radical approaches.

Frequency of environmental monitoring

I’m looking to assess our current EM frequency for non-sterile products (nasal an inhalation products). Areas are classified as ISO8/ EU Grade D for limits.
We currently monitor based on numbers of shifts. e.g 2 shift pattern we measure twice per month, 3 shift pattern we measure three times per month and im not in agreement with this and think we can possibly reduce to once per month or even less.

i cant see any actual documented guidance (USP,EP or Orange guide) that provides some example time periods that we can refer to support our decision.
If we decide that once a month or once a quarter is acceptable, who is to say that an auditor will agree and not have their own opinion. Therefore, I'm trying to at least have some basic frequency guidance to be comparing against.

FDA Reinforces Bcc Advisory

Just an FDA FYI and PSA re Bcc (long). This is what I have been seeing of late with FDA 483's and observations lately.

The FDA just published (07/07/2021) an advisory entitled: "FDA advises drug manufacturers that Burkholderia cepacia complex poses a contamination risk in non-sterile, water-based drug products".
Link: https://www.fda.gov/drugs/drug-safety-and-availability/fda-advises-drug-manufacturers-burkholderia-cepacia-complex-poses-contamination-risk-non-sterile?utm_medium=email&utm_source=govdelivery

The FDA advisory adds comments about additional contaminated materials found since their earlier 2017 findings ("Paroex(r) Chlorhexidine was recalled in 2020 due to objectionable microbial contamination including the BCC species B. lata.") of the original docusate sodium contamination finding in 2016.

In addition to the listing of products contaminated with Bcc the advisory also lists the CFR requirements that the agency references to ensure drug manufacturers are compliant with cGMP manufacturing of drug products. Of note are the following:

Sections on: BCC Growth and Contamination and Testing and Quality Risk Management

Of considerable interest is the following comment regarding Water Activity:
"Similarly, while drugs with higher water content are of most concern, deficient manufacturing or product integrity can lead to the introduction and survival of objectionable microbiological contamination in drugs with lower water activity.[12]"

1. ... assuring appropriate material quality, equipment and facilities, process design, maintenance and cleaning, production and storage time limitations, and monitoring of environmental conditions (21 CFR 211.113(a)).[9]
Of note is this reference reinforcing "objectionable": "[9]As noted in the preamble to the 1978 CGMP final rule, parts 210/211, "Microorganisms could be objectionable by virtue of their total numbers or their detrimental effect on the product or by their potential for causing illness in the persons ingesting them. A definition of the term is not practical in the regulations, however, because the objectionable nature of a microorganism may develop only in relation to the unique circumstances of a particular formulation, a particular ingredient, a particular method of manufacture, or the conditions found at a particular firm." 43 FR 45053."
2. Design, control and maintain a suitable water system, and ensure it does not provide conditions that support biofilm formation. Routine monitoring of microbial counts and identity of microflora....
3. Ensure vigilant production management and quality unit oversight ... This includes ensuring that distribution and piping systems used for water and drug manufacturing remain sanitary and suitable for their intended use.
4. Establish ... appropriate specifications and test procedures to assure that drug components (e.g., water and inactive ingredients) and finished drug products conform to appropriate microbial quality specifications ... This includes testing of raw materials and the finished product batch (21 CFR 211.165(b)) prior to disposition to ensure they are free of microbial contamination that is objectionable in view of the intended use of the drug.
5. Validation
6. Test in-process materials during the production process....
7. Investigate any failure to meet specifications or quality standards....

I'd like to hear any commentary on this specifically since Bcc provides a never-ending source of responses.

A1: This product, a Zinc Oxide Ointment for treating diaper rash contains 25% Zinc Oxide, lanolin, petroleum jelly and starch would not be considered a low water activity product, i.e., <0.6.

The recall notice on the FDA website states: FDA laboratory analysis confirmed that Pasta De Lassar Andromaco is contaminated with high levels of yeast, mold, and bacteria. The presence of Bcc in the product was not identified. No good supporting evidence from the FDA.

A2: Thank you for providing this link concerning this important advisory announcement from the FDA on Burkholderia cepacia complex contamination in non-sterile product formulations

It is interesting to see the water activity comment is referencing a product recall of a 25% Zinc oxide skin protectant for the treatment and prevention of diaper rash due to contamination with high levels of bacteria and fungi. It was a Mexican diaper rash cream sold in several US retail drug stores. Based upon this statement, it seems that FDA has a concern about the presence of Burkholderia cepacia complex in low

water activity products. They are correct that Burkholderia cepacia complex might not proliferate but are able to survive in low water activity products.

In addition, they are stating that routine identification of water borne isolates be conducted that had been obtained from routine testing. I have seen many companies only conduct identification of water borne isolates only after alert and action microbial levels are obtained for a sampling site of a purified water system. I'm not in agreement with this approach in conducting identification of water borne isolates only after alert and action levels are reached.

From a personal perspective, I do not have objections to the points that are made in this FDA advisory announcement about Burkholderia cepacia complex being a concern as a contaminant in non-sterile products.

A3: I do agree that using a reference of a 25% Zinc oxide diaper rash ointment product recall is not very supportive of indicating that low water activity formulations have a problem with Burkholderia cepacia complex contamination.

In general, it looks like the agency has a concern about Burkholderia cepacia complex contamination in all non-sterile product formulations no matter if they are high or low water activity product formulations. I would suggest that individuals interpret the possible meaning of this statement by the FDA even with a misleading reference on their own.

Furthermore, it is unknown to us what types of bacterial species had been isolated from this contaminated product that had been recalled in which high levels of microorganisms had been detected. Based upon information on the Red Alert Import List, I would not be surprised that Burkholderia cepacia complex was one of the contaminants, but the individual microbial contaminants of the high microbial levels had not been identified in the recall information.

A4: I try to express some of my considerations below.

The B cepacia problem is very complex and particular. First of all, they are a series of microorganisms and not all B cepacias isolated from the environment and components (water, products, raw materials, equipment ...) have the same ability to adapt and / or resistance to preservatives.
Here are some considerations:
- B cepacia can be added to the challenge tests at the time of the formulation study, but which one? because not all the isolated strains have the same resistance capacity, and also different from the ATCC strains. However, inserting an internal isolation B cepacia can be useful to avoid subsequent surprises. I also remember that the challenge assessment tables against bacteria are very different between EP and USP and, therefore, the level of protection of the formulas on the market is also different.
- working in a situation completely devoid of B cepacia is not easy and would also be expensive, so I do not think that ensuring an absolute absence of the microorganism is the correct way. However, remember that B cepacia already at 55-57 ° C for a time proportional to the charge to be removed, does not survive, therefore a possible treatment of water or bulk treatable in certain products can be useful.
- it is important, when we find microorganisms with a recurring trend on products that are not necessarily sterile, even if present in not very high quantities, to identify and verify their behavior over time within our products. This type of investigation is particularly important if the microorganism we find is of only one species. It is therefore important to check whether the detected charge drops, remains constant, increases over time.
- it is very important to worry about any pathogenicity towards the user, taking into consideration the possible state of health of the user to whom the drug is intended, but it is equally important to verify the damage that the microorganism can do to the formulation itself. If it survives or increases, it does so by feeding and this can modify the state of the product, altering its pH, appearance, forming precipitates, etc.

Some of these considerations are valid for all microorganisms considered "objectionable": on this denomination there would be many considerations to be made, first of all keep in mind that in our personal biome we have hundreds of different microorganisms.

A5: I know FDA is concerned with BCC and spore formers in commonly produced disinfectant products, wipes, and cosmetics. There should be a 483 report out there soon regarding this (and possibly a second notification of sorts by DOJ, shortly following). Having a close friend who's trying to survive long enough to become the oldest cystic fibrosis patient on record, I do not oppose FDA tightening its grip on these now heavily relied upon products in our society. Especially after seeing first-hand how loose that grip currently is.

A6: A number of clients have faced similar observations from the FDA dating back at least five years. In many cases the clients have upgraded or replaced their purified water (PW) systems to address BCC, only to find it returning at a later date. Since PW systems are normally run at room temperature, this may encourage the formation of biofilms over time; hot water systems have fewer incidents, although biofilm formation cannot be absolutely dismissed. For older PW systems a full assessment should be made to eliminate areas where biofilms may occur (e.g. dead legs, elbows, slopes, valves, connections, sampling points, etc.) and these areas should be remediated, redesigned and/or replaced. Once corrected my recommendation is to continuously monitor the PW system for the organisms and to perform comprehensive preventive maintenance of the PW to control the presence of these bacteria. Only by knowing exactly what the PW is doing can it be considered under control. One further comment: Engineers are very intelligent and design fabulous PW systems, however they do not always consider the microbiological implications of their designs. It would behoove a facility to contact a microbiologist to review proposed water system changes or installations, prior to performing the changes, to assure the design can maintain microbial control.

A7: I am in complete agreement with your comment about identification. I have seen companies that don't even ID at an alert limit. This has led to very difficult investigations, as in "we never see this organism in our water system" but they only have limited identifications. I am in favor of a routine monitoring of the water system and, at some frequency, identifying everything that is recovered.

A8: I would recommend you read a review article by my colleague Cheryl Platco.

My experience is adding the USP <60> B. cepacia to AET in general results in a more robust preservative system

AAPS PharmSciTech, Vol. 12, No. 1, March 2011

Comparison of Compendial Antimicrobial Effectiveness Tests: A Review Cheryl L. Moser1 and Brian K. Meyer1,2

Abstract. The antimicrobial effectiveness or preservative effectiveness test is described in the tripartite compendia for sterile parenteral multi-dose formulated products. The execution of the test is essentially harmonized with respect to inoculum preparation and test execution but not the acceptance criteria. This article describes how a single test can be performed that procedurally satisfies all of the compendia and their acceptance criteria.

KEY WORDS: antimicrobial effectiveness; pharmacopeia; preservative effectiveness.

A9: Thanks for starting this thread. The FDA's latest advisory represents a significant shift in Bcc enforcement from a focus on aqueous products with inadequate preservative systems that allow for Bcc to grow to highly infectious populations, to a focus on products formulated with purified water that may contain Bcc. It appears that Bcc, formerly considered a respiratory pathogen and particularly dangerous to immunocompromised and respiratory illness patients, will also be considered an objectionable organism in non-aqueous topical, vaginal, and transdermal products (similar to P. aeruginosa and S. aureus). Although there are certainly products specific for high risk patient populations delivered through certain routes of administration where Bcc may be considered an objectionable organism, (inhaled powders for COPD or other lung conditions, for instance), the vast majority of non-sterile non-aqueous products probably do not require the absence of Bcc. However, I do agree with the FDA's assertion that a thorough risk assessment should be conducted to identify the types of microorganisms that could be considered objectionable in the finished product.

According to the Objectionable Organism decision tree from PDA TR 67, if Bcc was found in a non-aqueous product, and that product did not list Bcc in its specification as an organism that must be absent from the finished product, the product would be considered acceptable for release. Because USP <60> correctly implies that aqueous and inhalation products are at highest risk for Bcc, few manufacturers list Bcc in their test specification for non-sterile non-aqueous products and to the best of my knowledge, the FDA does not consistently require manufacturers to do so. I understand the reason for the FDA's advisory, but I'm concerned about the consistency with which enforcement actions for Bcc in non-aqueous non-sterile products will be executed.

A10: The ointment cited in the recall is certainly non-aqueous as determined by the formulation published by the manufacturer that can be accessed by an internet search. The water activity of the product, the microbial count, and the range of microorganisms found in the product is not revealed. Based on water activities published for different dosage forms, B cepacia complex will not grow in an ointment. For a multiple-use product, the non-inclusion of a presentative system should have been provided to the FDA.

If this is representative of policy change, which I do not believe it is, there should be greater transparency and justification for the change.

A11: Your observations are 100% spot on, especially the commentary about the FDA consistency of enforcement! While this does represent an "official" shift in focus, it does follow the original impetus for the Bcc regulation - The 2011 Torbeck letter in PDA: Burkholderia cepacia: "This Decision Is Overdue" https://www.fda.gov/media/81867/download which noted the following:

"The evidence regarding the objectionable nature of this microorganism is substantial and supported by other independent research (29). Bcc organisms pose a clear and present danger to patient health and safety. The challenge is undeniable; now is the time to remove Bcc from our pharmaceutical manufacturing areas and products."

I do have concerns for many of the points, as you noted.

1. "Similarly, while drugs with higher water content are of most concern, deficient manufacturing or product integrity can lead to the introduction and survival of objectionable microbiological contamination in drugs with lower water activity.[12]". As you mentioned, I agree on looking at topical/vaginal/transdermal products and a thorough risk assessment should counter those regulatory questions. My fear is that this will potentially extend to solid oral dosage forms as well.

But my biggest concern is this comment from the advisory:

"2. Design, control and maintain a suitable water system, and ensure it does not provide conditions that support biofilm formation. Routine monitoring of microbial counts and identity of microflora...."

1. How do we monitor AND exclude biofilm and Bcc in purified water systems? As has been mentioned before on the PMFList, the vast majority of non-sterile purified water systems are cold water systems that are often difficult to keep as sanitized as hot water systems. Microorganisms will eventually proliferate as biofilms develop over time. Testing for heterotrophic plate count (and especially testing larger volumes of purified water than 1mL) should keep biofilm and Bcc in check, but the statement on requiring the identity of microflora in water (presumably even for systems that are under microbiological control) can become onerous and can get extremely expensive for those companies. USP <60> is specifically not for water (although you can make the argument as a raw material for some products), so adapting it to purified water monitoring looks to be difficult. Questions:
    * Have there been any studies looking at using BCSA and membrane filtration for Bcc in water?
    * Are there other media out there (chromogenic?) that could be used with membrane filtration and HPC counts for so that ID's would not be needed for every morphologically dissimilar colony?

* Are any of those media accepted by the Agency or will studies be needed to qualify the media with your water system. A note here - having tested hundreds of water systems, I have found that almost each one has is own unique microbiological flora, so media qualification may be needed for each system.
2. With the complexity of the Bcc species, will any micro testing be appropriate for this complex? As noted in one of the FDA advisory references (Jin et. al. https://biologydirect.biomedcentral.com/articles/10.1186/s13062-020-0258-5) The Bcc species seems to be growing from 22 species to possibly 36. How will this play out in future microbiological monitoring for non-steriles.

Great fun for microbiologists though!

A12: You are correct that microorganisms will not proliferate in an anhydrous product formulation, especially one that is petroleum based. However, you failed to mention that microorganisms will be able to survive in a petroleum-based product formulation. In reviewing this product recall, the reason for having high microbial levels in this formulation is unknown. For one, it is my understanding that Zinc oxide does have antibacterial and antifungal activity. Was the Zinc oxide encapsulated by the petroleum to prevent antimicrobial action? Is the presence of water necessary for Zinc oxide to have antimicrobial activity? Granted, there is no water in this formulation, but this is expected for most petroleum-based product formulations. Besides an error occurring during cleaning and sanitization of the manufacturing equipment of this product recall in which water was still present somewhere in the equipment, it is very possible that the lanolin and/or starch components of the formulation that had been used to make the product had been contaminated with high levels of microorganisms. In addition, I have seen anhydrous product formulations with low water activity readings, but they were still highly contaminated with microorganisms. A product recall is not just for a product in which organisms are just proliferating but whether they are also surviving in high numbers.

Regarding your statement concerning the non-inclusion of a preservative system in a petroleum based anhydrous product that is used multiple times, it is not unusual not to find the presence of a preservative. The inclusion of preservatives in anhydrous oil-based product formulations has been a subject of disagreement among microbiologists of whether to include or not to include for many years. If you did include a preservative in a petroleum-based product formulation, what challenge criteria would you use (e.g., reduction or statis)? At one time, the USP AET had challenge test criteria for anhydrous oil-based product formulations, and it turned into a disaster. If you wanted to perform challenge testing on an oil-based product formulation, there are many factors that need to be decided upon before testing is ever conducted ranging from which oil-soluble preservatives to include in the formulation, suspending fluid for each of the challenge test inoculums, preservative challenge testing criteria, etc.. In comparison to aqueous product formulations, it is not easy to conduct challenge testing on these types of oil-based product formulations. I will admit that most microbiologists performing challenge testing on product formulations do not have the knowledge in how to conduct challenge testing on oil-based anhydrous product formulations.

A13: 1. How do we monitor AND exclude biofilm and Bcc in purified water systems? As has been mentioned before on the PMFList, the vast majority of non-sterile purified water systems are cold water systems that are often difficult to keep as sanitized as hot water systems.

The best purified water system I encountered used ozone to sanitizer the storage and distribution system. Relative low ozone levels were generated and removed by UV light. However, members of the Bcc plageic or as a biofilm are quickly killed at temperatures around 60 degree C but the heating cycle takes the system out of commission and consumes energy.

Microorganisms will eventually proliferate as biofilms develop over time. Testing for heterotrophic plate count (and especially testing larger volumes of purified water than 1mL) should keep biofilm and Bcc in check, but the statement on requiring the identity of microflora in water (presumably even for systems that are under microbiological control) can become onerous and can get extremely expensive for those companies. USP <60> is specifically not for water (although you can make the argument as a raw material for some products), so adapting it to purified water monitoring looks to be difficult.

The assumption is that counts and the spikes will increase as biofilms formation occurs but daily or weekly monitoring is too infrequent to rapidly detect trends. Non-selective media such as plate count and R2A agar will isolate B. cepacia.

Questions:

* Have there been any studies looking at using BCSA and membrane filtration for Bcc in water?* I cannot recall any published studies*
*
* Are there other media out there (chromogenic?) that could be used with membrane filtration and HPC counts for so that ID's would not be needed for every morphologically dissimilar colony?* Yes, there is chromogenic media that enumerate total counts, P. aeruginosa and B. cepacia*
*
* Are any of those media accepted by the Agency or will studies be needed to qualify the media with your water system. A note here - having tested hundreds of water systems, I have found that almost each one has is own unique microbiological flora, so media qualification may be needed for each system.* Why not if they are qualified as suitable for use.*
*

2. With the complexity of the Bcc species, will any micro testing be appropriate for this complex? As noted in one of the FDA advisory references (Jin et. al. https://biologydirect.biomedcentral.com/articles/10.1186/s13062-020-0258-5) The Bcc species seems to be growing from 22 species to possibly 36. How will this play out in future microbiological monitoring for non-steriles. *With USP <60>, the three most clinically significant species were used for growth promotion testing. Pathogenicity is species and strain related.*

A14: I believe the underlying cause of the high microbial count in the Zinc Oxide ointment is the presence of starch in the formulation as water is not an ingredient

With a multiple-use product the key is the justification for not including a preservative, e.g., low water activity, self-preservation of the formulation, in-use microbial testing, delivery system, manufacturing process, etc.

In the distant past I did conduct lab testing that justified the removal of an antimicrobial preservative from a lip balm that used a hot filling process.

A15: FDA has routinely used USP <60> to confirm BCC in water samples it has collected from facilities when BCC has been recovered in product resulting in FDA's involvement (inspection, recalls, etc.). FDA has also used USP <60> to confirm the presence of BCC in process samples and finished product, as part of investigations to contaminated product in the field.

Therefore, you can use USP <60> to test water samples to confirm whether BCC is in the system, and this can continue at some periodic schedule to demonstrate your remediation efforts (if needed) and maintenance programs are adequate to prevent the growth of BCC.

I have been involved in numerous BCC remediation projects over the past year, and many are related to water systems associated with non-sterile manufacturing.

A16: They did write a sentence or two on testing water. There's a watered down TSB enrichment step prior to plating . It says "For Pharmaceutical Water", which has caused some confusion when it was initially released. FDA made it clear that this applies to nonsterile manufacturers only. That being said, it seemed clear that USP 60 would apply to aqueous, non—sterile MFG…..but it seems the FDA will apply to it anybody who has a Bcc problem. I guess if you're not at least looking, perhaps you should be testing your water periodically, even if you make a non-aqueous, non-sterile product. I'm not making a claim here, just thinking through what this recall and investigation could bring.

A17: My first choice would be a non-selective culture medium like R2A. B. cepacia may be readily isolated during the enumeration. USP <60> was written for non-sterile product testing but could be used for investigative purposes with water.

Growth Promotion Testing Requirements for R2A Agar (MerckMillipore) are as follows:

Organism	**ATCC**	**Inoculum CFU**	**Recovery**
Escherichia coli	8739	10-100	70-200%
Pseudomonas aeruginosa	9027	10-100	70-200%
Bacillus subtilis	6633	10-100	70-200%
Burkholderia cepacia	25416	10-100	70-200%
Ralstonia pickettii	27511	10-100	70-200%
*Methylobacterium extorquens**	NBRC 15911	10-100	50-200%
*Pseudomonas fluorescens**	NBRC 15842 17386	10-100	50-200%

*For slow-growing waterborne bacteria, store in purified water at 20-25°C for up to 7 days, inoculate and incubated for 4 to 7 days at 20-25°C. After JP 17 - Chapter 4.0.5, Chapter G8, Chapter G4

Query regarding GPT and suitability of BCSA

I have query regarding GPT and suitability of Burkholderia cepacia.

As per USP chapter <60> Microbiological Examination of Nonsterile Products—Tests for Burkholderia cepacia Complex, For GPT and suitability testing following three microorganisms are mentioned for Growth-Promoting and Indicative properties.

Can we use any one strain for Growth promoting and indicative properties while performing GPT and suitability testing please confirm.

Table 1. Test Strains of Microorganisms for Growth Promotion and Suitability Testing

Microorganism
Burkholderia cepacia (Growth-Promoting and Indicative)
Burkholderia cenocepacia (Growth-Promoting and Indicative)
Burkholderia multivorans (Growth-Promoting and Indicative)
Pseudomonas aeruginosa (Inhibitory)

A1: You have to perform for all 3 organisms as the chapter describes about Burkholderia cepacia complex.

A2: You test the medium with all three Burkholderia species.. The "or" in Table 1 is misleading. I've confirmed this with USP the intent of the text which is that all three species, not only one, are to be used for growth promotion and indicative properties of BCSA.

In addition, both P. aeruginosa and S. aureus are used to test the inhibitory properties of BCSA.

A3: All 3 strains of BCC are needed for the growth promotion test and suitability test.

Biological indicator - resistance performance tests <55>

From the text in the chapter, it seems to be that all end users/laboratories must perform all of the stated test independently from the manufacturer to confirm the results. Is it the case that we must perform all tests, especially the D-value determination? I was under the impression that D-value is a manufacturer test, is this noted anywhere else?

We currently perform population verification and identification.

A biological indicator (BI) is a well-characterized preparation of a specific microorganism with a known resistance to a specific sterilization process. The correct use of BIs in the development, validation, and control of sterilization processes requires that their population and resistance be accurately known. The population and resistance can be selected to confirm the adequacy of individual sterilization process

conditions for an article. The recommendations of Sterilization of Compendial Articles 〈1229〉<https://online.uspnf.com/uspnf/current-document/GUID-ECD53800-853C-40EB-9E08-F125CF4C999B_1_en-US> should be followed for effective BI usage. The methods described below can be used to establish population and resistance, such that the response of the BI to the subject sterilization process is appropriate. Although the BI manufacturers are required to maintain rigorous control of population and resistance using the number of replicates as specified below, the end users are not required to use the same number of replicates for verification of those determinations. Conduct all of the tests described in this chapter under appropriate microbiological laboratory conditions

Finding a contract lab that performs D-value test is quite difficult, costly and uses a lot of BI's. Having to independently verify the manufacturer info seems excessive.

A1: The text you are trying to recall was moved to USP <1229.5>. Here is the relevant text under section BI User's Responsibility:

"When a BI is used in accordance with the BI manufacturer's directions, the resistance of the BI need not be reconfirmed."

A2: When the BIs are used as per manufacturer recommendation, only verification of population is user responsibility. Please refer USP <1229.5>

BI User's Responsibility
When BI's are purchased, their suitability for use in a specific sterilization process must be established. The BI user should obtain a certificate of analysis for each lot of BIs and verify the manufacturer's label claims for spore population (see Biological Indicators—Resistance Performance Tests 〈55〉). When a BI is used in accordance with the BI manufacturer's directions, the resistance of the BI need not be reconfirmed.

A3: I believe that we have pointed to this statement to state the we did not need to perform D-value testing on our supplied BI's.

I am just making sure that we have interpreted it correctly and are ok in not performing the test. After re-reading again a few times it doesn't actually seem 100% clear that we don't need to do testing in line with the statement in <1229.5> by what is written in <55>

Although the BI manufacturers are required to maintain rigorous control of population and resistance using the number of replicates as specified below, the end users are not required to use the same number of replicates for verification of those determinations. Conduct all of the tests described in this chapter under appropriate microbiological laboratory conditions.

If an auditor just says to us that <55> is mandatory and <1229.5> is information, then how can we justify not needing to perform. I can't see anything in <55> that points to manufacturer only.

Any supporting statement would be greatly appreciated.

QC testing for ready to use inoculum

Just want know requirements and practices to qualify each lot/consignment of ready to use standard inoculum i.e. bioball and quanti cult.

What type of testing is performed for each lot? Whether identification upto species level is required?

A1: I would perform count verification and identification.

A2: Bioball and quanti cut are used pure culture suspension and yes for each lot / consignment enumeration and identification up to species level is required to confirm the label claim and purity of culture.

It's same like biological indicator.... For each BI lot or consignment we perform enumeration and identification up to species level.

A3: Identification up to species level once only for same lot of culture. Studies required for consignment to consignment lot Identification up to spp level. Draw conclusions as no changes in the Identification.

A4: We are following procedure for each received consignment of bioball/other cultures as per following:

count verification against the labelled claim with in house acceptance criteria of 70% recovery against vendor certificate mean count. For all bacterial strains in house identification up to species level.

For fungal strains only lacto phenol cotton blue staining. No identification up to species level, as no capabilities at site.

A5: We need to qualify each received lot should be confirmed by purity of culture and identify before use.

It is mentioned in the USP chapter 1117. Refer below statement from USP.

MAINTENANCE OF MICROBIOLOGICAL CULTURES Biological specimens can be the most delicate standards to handle because their viability and characteristics are dependent on adequate handling and storage. Standardizing the handling and storage of cultures by the user laboratory should be done in a way that will minimize the opportunity for contamination or alteration of growth characteristics. The careful and consistent treatment of stock cultures is critically important to the consistency of microbiological test results. Cultures for use in compendial tests should be acquired from a national culture collection or a qualified secondary supplier. They can be acquired frozen, freeze-dried, on slants, or in ready-to-use forms. Confirmation of the purity of the culture and the identity of the culture should be performed before its use in quality control testing. Ready-to-use cultures should be subjected to incoming testing for purity and identity before use. The confirmation of identity for commonly used laboratory strains should ideally be done at the level of genus and species.

Effect of Radiopharmaceuticals on bioburden / organism survival

Would anyone have a suggestion for a reference or direction to look in regarding the antimicrobial effects of a radiologic injectable on bioburden in general? This is a topic that I have not yet come across in Quality Micro, and am working with a product whose shelf life is shorter than the time it would take for the results of a 14d Sterility test to become available. What would the additional implications be (if any) on bioburden testing, and general sterility assurance concerns? I’m guessing not all radiologic elements have the same effect on the gamut of organisms that might find its way into an isolator, and am also guessing there are some organisms that would be resistant regardless.

A1: I have several clients that make this type of product. We found references for the specific radioisotopes on their likelihood of killing the microorganisms to aid in determining whether we needed bioburden testing.

A2: Your query is unclear as to final product sterility assurance or component bioburden (i.e. radiochemical /precursor or non radioactive adjuvant component supply). However let me put this concept of self-sterilization to rest. It is generally recognized in the radiopharma field that final drug product radiopharmaceuticals are not "self-sterilizing". Radiation fields encountered during radiopharmaceutical production and during the lifetime of radioactive drug products are not valid for achieving sterility assurance. This is has been a generally accepted position however it is based on historical predominance of diagnostic radiopharmaceuticals and their associated manufacture. The ascendance of new therapy radiopharmaceutical products may alter this paradigm but as of now this accepted position has not

changed. Your concern with the issue of drug product utilization and expiry preceding the final resolution of the recognized industry 14 day sterility test standard is a well known and recognized issue that the FDA has addressed in the unique PART 212 regulations and addressed in USP chapters 823 & pending USP 825. I would suggest you review the USP chapters for establishing a defensible approach to sterility assurance for radioactive drug production.

A3: Do you recall an example of a source where you located that information? I'm guessing the supplier of the radioactive material would not have that information on hand. I'm also guessing some suppliers of the radioactive material wouldn't be very giving with its internal research (but not sure..)

As a side note, I'm not sure FDA knows how to regulate these type of products. It seems 212 was written with a compounding pharmacy-type operation in mind. And it's written in a question / answer form (I just find that odd..). That said, if regulated under 212, which of the 211 regulations could be conveniently glossed over? Some 211 regulations would admittedly be more difficult to meet, and perhaps unrealistically. I see this as a bit of a quandary. This wouldn't be a bad topic at the next conference in my opinion.

The preamble of 212 mentions something to the effect that most companies that produce radiopharmaceutical products are likely to have some form of 211 (GMP's) already in place……. Not the most warm / fuzzy statement. More importantly, not the most clear.

A4: My query was a general one, as I'm trying to determine radiologic effects on microorganisms in general. Applicable to a radiologic product that undergoes final sterile filtration. Digesting 825 and 823 is on my imminent list. Thank you for the suggestions. I think a strong sterility assurance program is the item to focus on here.

A5: The D-value for vegetative microbial cells is <1 KGay while bacterial spores range from 1-4 kGay. I am not sure what the dosage is for a radiopharmaceutical but it is likely to be a lot less.

A6: One of the interesting things with radoopharmaceuticals used for treatment of hard tumors is that the physician ultimately sets the dose. From the manufacturing end, we can assure consistency and reproducibility, but in the end, if the physician wants to deliver 75GBq of radiation to a tumor at the beginning of treatment (and on a relatively otherwise healthy patient), that is his/her call. If a patient is weakened, or its the third dose, the physician may recommend a third of that dose.

An interesting twist indeed. They are basically "physicians cocktails" in the end.

A6: When looking for information on the killing power of radioactive materials on different cells, I researched articles available on academia.edu. I like that source, since so many articles are posted by the authors, without having to pay for them. Alternatively, you can ask the suppliers of the radioactive materials as well, if they have any safety literature.

Regarding the regulatory questions, some radiopharmaceuticals are regulated under 212, but there are also some regulated under 211. We were required to comply with things like the Aseptic Processing guidance for some of our products.

Covid-19 Testing Laboratory Standards

If a lab assistant position in a Covid-19 testing lab was proposed to you, what would you ask to the employer to understand if necessary safety guidelines are practiced?

I believe one must check for ISO 17025 certification but, should there be other specific requirements that has to be asked? (In order to protect employee safety).

Rounding Rules for MLT

We are in the process of setting up a LIMS system for data recording and the topic of rounding has come up and caused a bit of a divide between people on how LIMS should calculate final USP plate count results, such as TAMC.

Example: 1:10 dilution plated in duplicate, results are 4 CFU and 5 CFU. LIMS must calculate a reported result.

To start, I'm well aware and have already expressed to everyone that in the world of micro the difference between replicate results such as 4 and 5 CFU is meaningless, and the difference between 4.5 and 5 CFU are even MORE meaningless. To simplify discussion, let's not complicate the things even more with debate about 25 CFU LOQ. We are in splitting hair territory, but please bear with me.

On one side, we have proponents for LIMS rounding the mean result before applying the dilution factor. In the above example 4.5 CFU would round up to 5 CFU, and be reported as 50 CFU/g after applying the dilution factor. The argument is this reports a conservative worst-case scenario and CFU data should always be integers since you can't have a partial CFU.

On the other side, we have proponents for LIMS not rounding the mean and reporting the result as 45 CFU/g. The argument for this is USP General Notices says original values should be used for any additional calculations and that rounding, when required, is performed after final calculations have been completed. I suppose one could argue that 45 should be rounded to 50 anyway due to significant figures, but I'm not sure the LIMS system has the capability of determining dynamically how many sig figs to report. It likely must be set at a static value.

Yes, none of it really matters if specification is NMT 100 CFU/g. I would really appreciate a survey of PMFList contributors to see how you report results like this, extra credit if you can cite pharma guidance.

A1: USP General Notices states:

7.20. Rounding Rules The observed or calculated values shall be rounded off to the number of decimal places that is in agreement with the limit expression. Numbers should not be rounded until the final calculations for the reportable value have been completed. Intermediate calculations (e.g., slope for linearity) may be rounded for reporting purposes, but the original (not rounded) value should be used for any additional required calculations. Acceptance criteria are fixed numbers and are not rounded. When rounding is required, consider only one digit in the decimal place to the right of the last place in the limit expression. If this digit is smaller than 5, it is eliminated and the preceding digit is unchanged. If this digit is equal to or greater than 5, it is eliminated and the preceding digit is increased by 1.

Rules for plate counts are discussed in the FDA Bacteriological Analytical Manual.

A2: I'd already reviewed FDA BAM Chapter 3 APC but it doesn't help much applying BAM plate count rules to USP since it says to report plates with fewer than 25 CFU as <25 CFU × 1/d, which is what my LOQ reference below was about. In any case, BAM APC text doesn’t discuss rounding other than to round to two sig figs and use the odd-even rounding rule rather than traditional rounding rules for numbers ending in 5 (which is in direct contrast to USP rounding rules).

USP General Notices 7.20 text is what I was referring to below on the debate side of not rounding until all calculations are done. Does this mean you support no rounding that .5 CFU result before applying dilution?

A3: Robert if you count a plate prior to rounding you never see 0.5 CFUs.

So with an example of duplicate plate counts of 4 and 5 CFUs, you average and get 4.5 and round up to 5 CFUs.

With analytical methods you report the result to the degree of accuracy of the specification.

SDA

I have an environmental monitoring program that currently uses TSA exclusively. The use of TSA has been verified to show adequate growth of fungi. Additionally we have performed studies that have demonstrated the equivalence of using TSA at 25 C to SDA for the detection and enumeration of fungi. Would there be any benefit to the addition of periodic use of SDA for fungal detection?

A1: Nope. TSA is more than adequate.

A2: As you can recover mold on the TSA plates I see two options - one periodic SAD monitoring or two adding SDA for a limited duration if you get mold on the TSA plates.

A3: Periodic use of SDA would be a waste of time and effort, not too mention it would call into question why a firm needs to utilize it sporadically.

I would agree if you opt to use SDA to support a mold related investigation. Then you can make a case for its limited use.

Other than that, no need for SDA on a routine or on any rotational basis.

A4: Yes I would favor using SDA for investigative purposes.

A5: A number of common molds die in the temperature range 27-32C so just using a TSA media @32.5C would have the risk of running blind to a possible excursion. Is that a significant issue?

A6: If that is a concern in a firms' environment they can incubate TSA at the appropriate lower temp.

Didn't want to bring temps into the discussion after I already kicked the beehive! That's next weeks' happy hour discussion.

I look forward to temp validation discussions. That usually gets us all amped up.

A7: This would not be an issue with our program. We incubate the TSA at two temperatures. First at 20 - 25°C 5 -7 days - then 30 - 35°C for 3 - 5 days.

Media fill failure

Aseptic process simulation failure is probably one of the biggest problems in industry and a huge challenge to find root causes. If you think you have found the root cause (improper tank valve closure of a tube in contact with drainage tube) of an staphylococcus-based contamination and you repeat the media fill batch (after implementing corresponding CAPA) and you get a new failure with a different contaminant (gram positive rod)... Do you think it is probable to be due to a different cause?

Have you ever had media fill failure? Which was the root cause? Any shared experience or advice is for sure useful!

Aseptic Media Fill biannual requirement

We are in the development/submission batch stage with our current 2 products. I am curious what others use for their twice a year media fill requirement. Do you give your site a leeway of 1 month, 2 months, etc?

Detection of E coli (Method suitability)

in case of detection of specified pathogens in non sterile products prepare the sample by taking 10 g products in 90 ml scdm or any other suitable diluents. in test of e coli we take 10 ml (equivalent to 1 g sample) from this preparation and inoculate in suitable amount of scdm and incubate at 30 to 35 temp for 18 to 24 hours. because criteria of e coli is absent per g as per pharmacopoeia. my query is for detection of E. coli in presence of product or method suitability.

Can we add 100 cfu of E. coli in initial preparation of sample i.e 10g sample in 90 ml scdm or add after taking 10 ml from this sample preparation.

Please justify.

Microbial recovery in presence of product

As per USP:

We take 1:10 dilution i.e 1 g sample in 10 ml diluent (total volume) and diluent is SCDM.

Prepare the microbial suspension of culture not more than 100 cfu.

1. Add to the sample prepared above a sufficient volume of the microbial suspension to obtain a inoculum of not more than 100 cfu. what is the meaning of this point.

Direction - in my opinion add 0.1 ml volume containing 100 cfu in above prepared sample and recover organism by pour plate method by pouring 1 ml on plate, 10 cfu per plate or 100 cfu in whole inoculum (sample +diluent+ culture) or

add 0.1 ml volume containing 1000 cfu in prepared sample and recover organism by pour plate method i.e 100 cfu.per plate. which is appropriate.

2. in case of surface plate method we take 0.1 ml sample with inoculum and spread on surface. if we add 100 cfu in above prepared sample. there is no probability to recover microbes. how much take inoculum for this method.

3. in case of membrane filtration we add 100 cfu in above prepared sample and filter whole qty and recover 100 cfu.

Please justify we should use same inoculum for three methods or inoculum maybe change.

Media pH

We are using dehydrated culture media for microbiological analysis in which media pH is already set by manufacturer & they also mention the range of media pH for the acceptance (For example: X media has pH 7.00,Range:7.00 ± 2). If the media pH is diverge from the given range than it can be set by any chemical or reagent ? Is there any standard acceptance limit for the given range?

A1: Yes, you can add 1 N NaOH or HCL to adjust the pH accordingly. Here are our suggestions for adjusting the pH:

"The pH should be corrected by adding 1N or 1/10N hydrochloric acid or sodium hydroxide solution to a sample of known
volume taken from the reconstituted culture medium (e.g. 50ml). The volume of acid or alkali added to the sample can then be used to calculate the quantity necessary to adjust the pH of the remaining prepared

culture medium. Therefore, it should be kept liquid during the pH measurement of the sample, in order to be adjusted if necessary."

A2: We have encountered this issue only with one type of media which is not part of those compendial media. We could not get the pH range that was recommended by manufacturer- our pH range was slightly lower than the specified range. In this case based on the growth promotion results, we rationalized the use of media as all the GP test criteria was within the acceptable range and requirements.

A3: Reading this conversation, I have a doubt in the case of agars how can we make ph adjustment taking into account that the prepared media solidify when it reaches 25°C?

A4: For adjustment of pH of solidified media, we pre-assumed about the drop before autoclaving and adjust the pH of liquid media accordingly. Once the media is autoclaved, poured into plates and solidified, we use a flat bottom pH probe to check pH.

A5: You change the pH while it is in liquid form, before autoclaving.

A6: Kindly provide BET methodology for the L Cystine HCl raw material,

A7: I think the concern was that rehydrated media will generally be above 50 deg C and that causes the issue of temperature compensation in the pH measurement if your meter was calibrated with buffers at room temperature. I'm not an expert on this topic, but I would say either use a qualified pH meter with ATC capability (automatic temperature compensation) or you'll have to standardize your meter with buffers at approximately the same temperature as the molten media you are measuring. Then, as others have said, adjust the pH while the media is in liquid form before autoclaving.

A8: But if the ph measurement and adjustment must be done in a portion of culture media which have to have a temperature of 25°C according to manufacture instructions, in this condition the media is solid. How can i adjust the ph in this portion?

A9: For that we have to use flat pH probe electrode which are available in the market under various brand.

A10: The best practice for starting testing any new material would be to perform inhibition and enhancement testing on the material. Even if I had a procedure for you the test for non-interfering dilution should still be on record at your facility. The test for non-interfering dilution typically involves testing a series of dilutions of the material using BET water. For a complete unknown I typically start at neat and do 1:10 dilutions to 1:10,000. This will typically find the point where product interference is overcome. You can come back with smaller dilutions later if needed find exactly where interference is overcome. If this dilution is beyond maximum valid dilution (MVD) then other methods of overcoming interference may be required such as, using a buffer for dilution, adjusting pH or other methods. Depending on your testing method (Chromometric, Turbidimetric, Gel Clot or a recombinant method such as Pyrosmart Next Gen) the non-interfering dilution could range widely so it's very difficult to say where to start.

Autoclave

Please help - Out of the two autoclaves in the lab(one is for media sterilization and the other for decontamination), the media sterilization autoclave has malfunctioned.

The repairs and spare parts are going to take a while due to the covid regulations.

What I'd like to know is can I use the autoclave (for decontamination) for media sterilization after I do a risk analysis?

What are the general guidelines in a situation like this?

A1: To use a decontamination vessel for media sterilization one would have to validate the appropriateness of the cycle. In addition to sterilization, you want to make sure you can sterilize without degrading the media. You also need to be able to remove the media without compromising the sterility. It might be easier to buy sterile media.

A2: Obvious issues are the validation status of the waste autoclave in terms of sterilization of microbiological media, the slow exhaust and media cool down capacity, the listing of the autoclave in the establishment license and the possibility of contaminating the media from the waste material or the steam generated to run the autoclave.

A3: If you're using a decon (overkill) cycle for media sterilization, you run the risk of denaturing the proteins in the media that is needed for organisms to grow. I'd personally avoid it if possible. I've recently been on an FDA audit where this was directly challenged.

A4: If your decontamination autoclave not having validated load pattern for media preparations then raise deviation first. As a CAPA you may propose to validate media preparations cycle in decontamination autoclave. Then yes in the event of malfunction in media preparations autoclave, you may use decontamination media, but ensure risk assessment. Before media preparations cycle an empty cycle is to be taken, and extra cleaning of autoclave to be done after completion of decontamination cycle.

A5: Help me understand how a decontamination cycle (waste/biohazard) could contaminate a subsequent media cycle? Both loads are sterile at the end of the cycle. How is it possible?

I've been asked by auditors also to dedicate one unit to media and one to waste and I've argued against it. I validated both of our units exactly the same- so media and waste loads could be run in either one, and we have no issue if one goes down for repair. Also, the wear and tear on the machines is similar.

Microbiological assay of amphothericin b

In USP, potency of amphothericin b is not less than 750 microgram per mg on dried basis.

We have api of amphotericin b to perform microbiological assay.

We have USP ref standard of 994 mcg per mg on dried basis and follow instruction before use dry.

We get result 1028 mcg per mg on as is basis.

Is it possible that result is exceed 1000. as 1 mg equal to 1000 mcg. and on dried basis is become more due to loss on drying?

2. In British Pharmacopoeia potency of amphothericin b is mentioned not less than 750 IU per mg on dried basis. in this connection 1mcg equal to 1 IU or conversion factor apply to change mcg to units.

3. IU may be cross 1000 but not in case of microgram.

4. We should use same standard i.e if result show in units per mg than standard use units per mg or if result show in mcg per mg and use std mcg per mg. or conversion factor is best way?

ISO 5 sampling in ISO 7 environment

I am working with a client who intends to add a personnel monitoring site for an operator in the ISO 7 background supporting operations in the BSC. This operator does not participate in manipulations within the BSC, and enters solely up to the wrist to pass items in to the BSC technician. The intent is to sample ISO 7 operator's gloves as follows: -Operator performs tasks in the ISO 7 space (documentation,

equipment./computer data entry, etc) -The operator performs glove sampling in the ISO 7 space -The operator then sanitizes gloves and materials immediately prior to transfer/entry into the BSC.

The site is planning to hold this sample to ISO 5 limits, with the argument that the ISO 7 operator's hands are always 'ISO 5' because they support material transfer into the BSC.

I would appreciate any input on this approach, as it is not in line with what I have seen in the past. Have other sites sampled the support operators during operations in this way, or would an alternate approach/ standard be appropriate?

A1: From the way you described this, it would be more appropriate for the criteria of these support operators to be consistent with ISO 7, or Grade B glove print (Grade B glove print level is 5 CFU). You cannot expect a person grasping materials and items that are not rendered sterile on the outer surfaces (but wiped down, usually IPA) to obtain expectation of 0 CFU or no-growth. Furthermore, you said these people do not participate in manipulations within the BSC.

With that said, you can consider that a any CFU recovery from these support operators may consider at least genus level identification so that any recovery within the limit is not just ignored. Or, you could say that any recovery signals an alert to watch. If say you had repeated recovery from these glove prints of 3 consecutive hits or more, although all within the 5 CFU limit, you would then consider this an unfavorable trend. This would mean that the wipe down procedures are not doing very good job.

MLT test quantities

I have to test a non sterile lyophilized product for microbiology (MLT).
USP <61> test is10 gram of product.
For this product,
Each vial contains 100 mg powder.
The batch run is 4000 to 8000 vials.
The resuspension volume is 1-3 mL.
The product is expensive.

How many vials do I have to test?
How do I justify this ?

A1: Refer to the "Testing Of Products" section of USP <61>.

You have between 400g and 800g total of product. It tells you to test 1% of the batch.

A2: Please find the below paragraph of the same USP <61>

image.png

Sterility test of sterile API

I have question related to the sample quantity used for the sterility test of sterile API. I refer USP 71 but didn't get clarity.
Please guide me.

A1: You can determine your sample testing volume in method suitability test. When comes to bulk sterility testing . NMT 10% of sample is used to inoculum medium.

USP 81 Assay Variability

We have an in-house cylinder plate method developed for the Amphotericin B. Testing the same lot on different days gives us high variability (We do three independent runs and take an average). Mostly because of the diffusion problem. Each plate has different sizes zones.

Are there any tips for reducing the variability in USP 81 plate-based assay? Also, What should be the specification limit for Lyophilised Amphotericin B Liposome for Injection? Currently, we have 90-110%.

Bioburden sampling of a radiopharmaceutical sterile injectable

It is proposed that Bioburden sampling need not be performed on a radiopharmaceutical sterile injectable. During formulation the drug substance volume is less than 100mL (its between 50 and 75mL). The arguments are 1) its a closed system within an isolator, and cannot open the system to sample (there are safety concerns here as well) and 2) the formulation size is less than 100mL and results are reported as greater or less than 10mL per 100mL. If the entire solution is less than 100mL, this would make taking a 100mL sample impossible.

Is the justification for not performing bioburden sampling here sound in others' opinions here? Its worth noting that it was shown that the radioactive material causes lethality within 30 hours of end of production (filling).

A1: Proposed by whom?

A2: The world of radiopharmaceuticals is strange for someone with a background in sterile production. Not all the tests that you do in regular production are possible.

Pre-filtration bioburden is not feasible and does not provide useful release information. The product is injected into a patient within a few hours of production.
Read the chapters in the USP for PET.

A3: This was proposed by a client.

A4: Yes I hear you - shelf life is less than one week…

Would PET regs apply to a beta-emitting product, though?

A strange field indeed, but extremely promising for cancer treatment. I think PMF should have a session on this field in an upcoming seminar. At the end of my current effort, I may be in a position to speak on this myself.

A5: Radiopharmaceuticals are radioactive at production time. How would you do bioburden? Membrane filtration? Not safe. If you wait till decay to safe levels the bioburden may have changed.

A6: To answer this question best to evaluate what regulatory compliance arena you are working in. Radiopharmaceuticals are a complex area, they do not enjoy simple regulatory oversight as they can be manufactured under FDA PART 210/211, Part 312, PET carve out under Part 212, or compounded under 203B and/or USP 823 under Practice of Medicine/Pharmacy, each brings its own level of regulatory compliance with respect to Bio-Burden. As always ALARA (As Low As Reasonably Achievable) principles are paramount and FDA is cognizant to this issue. From the sound of it (volume) your product seems to be a compounded drug product, and as such Bio-Burden studies on the final product should not be expected, not excluding final product sterility testing. A defensible approach for radiopharmaceuticals is bioburden assessment of "cold" components and starting materials along with documented production environmental controls along with 14 Day end product sterility testing data from your validation runs.

microbial examination of non sterile products

In testing of microbial examination of non sterile products, we prepare sample by taking 10 g product in 100 ml diluent and make up dilution 1:10. if product is not soluble or partially soluble in diluent and settled down, can we take supernatant from sample preparation tube for TAMC testing. we have already add polysorbate 80 and neutralizer in diluent. if taking, is there any documented reference.

A1: Some of my past radiopharmaceutical customers inoculated septum vial FTM and TSB with the product they produced. The downfall is that the patient still receives treatment before the QC results are finalized.

Marsh Periwinkles and Fungiculture

I was surprised to learn the *Littoraria irrorata, a* marsh-dwelling marine snail* i*nfects the tall marsh grass *Spartina alterniflora which grows in salt marshes on the east coast of the US *with fungus which it subsequently returns to eats as high caloric food.

As a microbiologist I was aware of so-called fungiculture by ants, termites, and some beetles but not a snail.

I plan to look next time in Marshland Conservatory in Rye, New York.

Overcoming Gentamicin and Sodium Azide Inhibition

I'm working on method development for USP <61> and <62> for a product that contains Gentamicin and Sodium Azide. Do you have any suggestions for enrichments or additives to help overcome this inhibition?

A1: Use a membrane filtration method and rinse the membrane.

A2: You can use a PVDf filter that won’t bind to the antibiotic.

A3: If you are trying to neutralize the antimicrobial activity of Gentamicin and Sodium azide in a non-sterile product formulation, the antimicrobial activity of Gentamicin can be neutralized by using Sodium nitrite. Sodium nitrite will prevent bacteria from uptaking this antibiotic. The reference for this is as follows:

Zemke, A. C., Gladwin, M. T., & Bomberger, J. M. (2015). Sodium nitrite blocks the activity of aminoglycosides against Pseudomonas aeruginosa biofilms. *Antimicrobial agents and chemotherapy*, *59*(6), 3329–3334. https://doi.org/10.1128/AAC.00546-15.

I'm assuming that you are using Sodium azide as a preservative because it is common to use Sodium azide as a bacteriostatic preservative at a concentration of 0.02% in aqueous laboratory reagents and biological fluids containing proteins. I do know that nitrous acid will neutralize Sodium azide. Because I have not worked with Sodium azide as a preservative due to safety and toxicity issues, I'm unaware of any other chemical neutralizers for Sodium azide that can be used in a microbial count for antimicrobial activity neutralization.

Besides membrane filtration that [name redacted] had suggested, it might be possible to neutralize the antimicrobial activity of Gentamicin and Sodium azide by using a microbial count diluent containing Sodium nitrite to neutralize the antimicrobial activity of Gentamicin and physical dilution to neutralize the antimicrobial activity of Sodium azide.

A4: The most common neutralization options include the following:

a) adding an antibiotic neutralizing/inactivating agent (usually enzymatic) to the broth/agar recovery media

b) increasing the concentration of the antibiotic neutralizer/inactivator in the broth/agar recovery media

c) physical separation of the organisms from the antibiotic (i.e., utilizing membrane filtration). Use an alternate membrane filter media (e.g., polyvinylidene difluoride [PVDF] or polyethersulfone [PES]), as cellulose-based membranes tend to bind antibiotics. If the organism does not grow on the antibiotic-filtered membrane, there is still activity within the membrane (assuming that the membrane material itself was checked for antimicrobial activity in the absence of the antibiotic). In addition, if the antibiotic is still bound to the filter membrane, it may be released upon introduction to the recovery medium. As part of your validation protocol, you should add a low number of organisms directly into the filter funnel prior to the final rinse. The antibiotic inactivator should have contacted the filter for a predetermined time prior to addition of the inoculum.

d) increase the volume of the rinse

Your best option is probably a combination of the membrane filtration method with enzymatic/chemical neutralization.

At some point in the process, you may need to state that the material under consideration has enough intrinsic antimicrobial activity to warrant modification or exclusion from <61>/<62>. You may want to incorporate the following statement to support data for organisms not recovered during your validation exercise: "the product is inimical to the low-level microbial challenge (< 2 logs) and that low numbers, if present, would be injured/killed and would not be recovered using standard test methodologies described in USP <61>/<62>."

Be careful to include appropriate controls in your methods validation because there is evidence of some materials binding to certain membrane filters. Post-incubation recoveries are then compared against one another.

Control #1 = Agar + diluent + membrane
-Tells us whether the organisms can be recovered.

Control #2 = Agar + diluent + membrane + antimicrobial neutralizing chemical(s)
-Tells us whether the organisms can be recovered in the presence of the neutralizing chemical(s).

Control #3 = Agar + diluent + membrane + antimicrobial neutralizing chemical(s) + raw material or product
-Tells us whether the organisms can be recovered in the presence of actual product.

References:

Corry, J.E.L., Van Doorne, H., Mossel, D.A.A. (1977) "Recovery And Revival Of Microbial Cells, Especially Those From Environments Containing Antibiotics", In: Antibiotics in Agriculture, M. Woodbine (ed), Chapter 12, pp.174-196.

Russell, A.D.; Ahonkhai, I. and D.T. Rogers. (1979) "Microbiological Applications of the Inactivation of Antibiotics and Other Antimicrobial Agents," J. Appl. Bacteriol., Vol. 46, pp. 207-245.

Breeze and Simpson. (1983) "Actual and Potential Methods ofTesting Antibiotics." In: Antibiotics: Assessment of Antimicrobial Activity and Resistance, ed. by Russell and Quesnel, Academic Press, pp. 339-348.

Russell, A.D., and Rogers, D.T. (1984) "Laboratory Uses of Antibiotic-Inactivating Enzymes", J. Antimicrob. Chemother., Vol. 14, p. 567.

Miles, A.A. and J.P. Maskell (1986) "The neutralization of Antibiotic Action by Metallic Cations and Iron Chelators," J. Antimicrobial Chemotherapy, April, Vol. 17, No. 4, pp. 481-487.

Miles, A.A., Maskell, J.P. and S.J. Evans (1986) "The Quantification of the Neutralization of the Action of Antibiotics by Cationic Iron," J. Antimicrob. Chemother., August, Vol. 18, No. 2, pp. 185-193.

Negretti, F. (1989) "Experimental Observations on the Bacteriological Controls of the Antibiotics--I. Antibacterial Activity of Membranes Employed in Bacteriological Assays," J. Pharma. & Biomed. Anal., Vol. 7, No. 12, p. 1861.

Casetta, P. and F. Negretti (1992) "The Microbial Controls of Antiseptics and Disinfectants by Membrane Filtration: Experimental Evaluation of the Interferences," Boll. Chim. Farmaceutico, Vol. 131, No. 6, pp. 238-241.

Specification for B. cepacia in purified water

I need to determine a specification for B. cepacia in purified water. What is industry standard? Are firms requiring absence in the purified water or is there a numerical limit?

A1: Nicole there is no specification for B. cepacia in Purified Water, USP. According to the literature B. cepacia may be frequently isolated from purified water.

The recommended limit for total aerobic microbial count is NMT 100 CFU/mL. There is a GMP requirement to exclude objectionable microorganisms from non-sterile drug products. B. cepacia would be considered objectionable in many aqueous oral, topical, nasal and inhalation products.

You need to mitigate this risk.

A2: No need for a BCC quantitative limit. Just go with your current CFU/mL spec. As for testing for the absence of BCC, that will depend on a number of factors. For example, is the water used to make non-sterile, aqueous dosage forms that may be used by immunocompromised, very young or elderly patients or consumers? Is the water system well maintained? Have you ever recovered BCC from the water system? These are just a few of many questions to answer to determine if you should be testing for BCC on a routine basis. Michael

A3: [name redacted] is correct that there is no USP specification for the absence of Burkholderia cepacia complex in purified water. In addition, the USP does not require the absence of any other specified organism in purified water. The USP only has an enumeration limit for purified water. However, a regulatory authority will always question you as to which specified organisms are considered to be objectionable in your purified water system. From my perspective, it is better to have the absence of all Gram-negative bacterial species than just having a list of organisms that are objectionable in samples of a purified water system. Burkholderia and Pseudomonas species are not the only Gram-negative bacteria that would be considered to be objectionable in a non-sterile products and purified water system. Besides these 2 Gram-negative bacterial genera, Ralstonia and Sphingomonas species are also commonly found in purified water systems which would also be objectionable if present in a non-sterile aqueous product formulation.

A4: We have been seeing an increase in Sphingomonas species in our water system (the CFU's have been over our specification limit at times). I haven't found many articles indicating that Sphingomonas creates biofilms and was wondering if anyone else had some insight into this? Also, what chemicals can be used to eliminate Sphingomonas once it is in your water supply?

A5: Therefore the (rhetorical) question is, how do you eliminate all Gram negative rods from purified water systems?

A6: It is well known that Sphingomonas will form a biofilm. Attached for your reference are several articles indicating that Sphingomonas will form biofilms:

Gulati P, Ghosh M. Biofilm forming ability of Sphingomonas paucimobilis isolated from community drinking water systems on plumbing materials used in water distribution. J Water Health. 2017 Oct;15(6):942-954. doi: 10.2166/wh.2017.294. PMID: 29215358

*Azeredo, J., and R. Oliveira. 2000. The role of exopolymers in the attachment of Sphingomonas paucimobilis **Biofouling **16:59-67.*

*Gusman, V. et al. 2012. *Sphingomonas paucimobilis as a biofilm producer. Archives of Biological Sciences <https://www.researchgate.net/journal/Archives-of-Biological-Sciences-0354-4664> 64(4):1327-1331. doi:10.2298/ABS1204327G <http://dx.doi.org/10.2298/ABS1204327G>*.*

*Stiina Rasimus, Marko Kolari, Hannu Rita, Douwe Hoornstra, Mirja Salkinoja-Salonen, Biofilm-forming bacteria with varying tolerance to peracetic acid from a paper machine, **Journal of Industrial Microbiology and Biotechnology**, Volume 38, Issue 9, 1 September 2011, Pages 1379–1390, **https://doi.org/10.1007/s10295-010-0921-4 <https://doi.org/10.1007/s10295-010-0921-4>.*

It is not that uncommon to isolate Sphingomonas in a purified water system. See the following article: Sandler, T. 2015. Characterizing the Microbiota of a Pharmaceutical Water System: A Metadata Study. SOJ Microbiol Infect. Dis. 3 (2): 1-8.

From my experience, it is very difficult to remove a microbial biofilm consisting of a Gram-negative bacteria after establishment. I have found that chemical sanitizers/disinfectants are ineffective in removing a biofilm. From my experience, the most effective way to remove a biofilm in a purified water system is to use a heat sanitization step by using circulating hot water.

A7: There are many articles that talk about B. cepacia and biofilm. I am attaching one to you.
The best way to eliminate this family of microorganisms is by heat treatment of the distribution loops, starting at 60 degrees. You have to do some tests to check if the treatment goes well in all points and to establish the time necessary to break down the biofilm.

A8: Yes it does produce biofilm.
Biofilm forming ability of Sphingomonas paucimobilis isolated from community drinking water systems on plumbing materials used in water distribution - PubMed

Biofilm forming ability of Sphingomonas paucimobilis isolated from commu...

Sphingomonas paucimobilis, an oligotroph, is well recognized for its potential for biofilm formation. The presen...

As with most microorganisms that are responsible for biofilm formation in a water system, simple disinfection/chemical removal may not be sufficient. Since there are many areas within a system where organisms can be confined, such as filters, pumps, pipes that are improperly sloped, elbows, etc. etc., it is important to first find out where in the system they might have a biofilm party. It's not easy and takes time. Feel free to contact me if you would like additional information.

A9: "From my perspective, it is better to have the absence of all Gram-negative bacterial species than just having a list of organisms that are objectionable in samples of a purified water system."

Im sure that most of us agree with Don but in the real world, this doesn't seem possible; e.g., given the age of the various purified water systems, presence of physical factors like sanitary 3A fittings, proper Reynold's Number for water flow, and on and on and on.

The name of the game is CONTROL.

A10: Think you can expect this and about any Gram negative to develop biofilm. Is the issue associated with distribution loop(s) or generation? Perhaps a caustic rinse would be helpful.

A11: Heat does not eliminate biofilm - tho' it may kill the bugs offering transient quality. There's a reason the micro issue developed and remnant biofilm will speed the recurrence of the issue.

A12: That is exactly what Gram negatives do because of their cell wall composition. Generally you will find a biofilm growing within minutes due to short generation times.

A13: You have to remember that the source of Gram-negative bacteria in a purified water system will be from one or more of the following sources: in-coming feed water, sand beds, carbon filters and cation and anion exchange beds. There are many factors that are involved in designing a proper purified water system.To get rid of Gram-negative bacteria in a purified water system, it comes to having a properly design of the system such as having no dead-legs in the distribution loop and whether it is either a hot or ambient temperature circulating purified water system. If the system is a hot circulating system, it is highly unlikely that Gram-negative bacteria would be isolated in the distribution loop that are commonly found in ambient temperature purified water circulating systems. For ambient circulating purified water systems, these are the problem systems. To establish control of these ambient systems, you will need to have the installation of proper microbial control measures such as micron cartridge filters and ultraviolet lights in the generation portion of the system if you are not using ozone in the distribution loop to control the presence of organisms. I will admit that I like to get rid of cation and anion exchange beds and instead use a continuous electro-deionization unit because microorganisms do not proliferate in these units and they also remove cations and anions.

Since becoming a consultant, I have seen many poorly purified water systems that are in use by many companies that have major design flaws that make them nearly impossible to be free of Gram-negative bacteria. I'm shocked that these systems are still present in the industry after the FDA had issued their guidance document on purified water systems in the 1980's.

I have found that the best references in having a properly designed purified water system are as follows:

Soli, T.C. Design and sanitization of water systems to prevent contamination. In: Designing and Controlling Water Systems (Madsen, R.E., and Moldenhauer, J., eds.) Parenteral Drug Association, Bethesda, Md. 2014.

Collentro, W.V., Pharmaceutical water system design, operation, and validation. Second Edition. Informa Healthcare. Interpharm/CPC., New York, New York. 1999

Meltzer, T. Pharmaceutical Water Systems. Tall Oaks Publishing, Inc. Littleton, Colorado. 1997.

A14: I'm curious about the position that all Gram negative species are objectionable. Wouldn't that essentially require a water system to be sterile?

A15: I have found that flushing the system 3 times with a Minncare solution, making sure to flush it out all the ports, will beat back the biofilm for a while. You would of course, have to determine if that chemical is appropriate for your system.

A16: In my opinion, establishing genus-based criteria for objectionable organisms is a very conservative approach that could lead to the unnecessary rejection of good product. Although there are certainly pathogenic strains of Pseudomonas, Sphingomonas, and Ralstonia that would be considered objectionable in aqueous drug product-, there are also non-human pathogens like Pseudomonas syringae, Ralstonina solanacearum, and Sphingomonas elodea that may be present in purified water systems or product. Those organisms would not be considered objectionable, or even organisms of concern, if isolated from aqueous drug products because they do not cause disease in humans. A risk-based approach that considers the product’s route of administration, the intended patient population, and the pathogenicity of the isolated organism is a better approach for determining if an organism should be considered objectionable. I highly recommend speciating the organisms isolated from raw materials or finished non-sterile products before making any decisions on objectionability.

A17: Don't folks address water system status via Alert/Action level considerations than specification?
I agree with [name redacted] - detection of Gram negative bacteria, esp. repeated, in water, product, product contact EM, etc. clearly risks product quality.

A18: I totally agree with [name redacted] position that we should direct our objectionable organism screening to non-sterile drug products and mitigate risk.

Your risk mitigation steps should be based on the reality we cannot totally exclude Gram-negative bacteria from purified water. According to a book chapter from Tim Sandle (2015) he identified 315 bacteria from a purified water system from 2000-2014 with the most prominent genera Ralstonia (30%), Buckholderia (23%), Pseudomonas (9%), Moreaxella (7%), Flavimonas (5%), Stentrophomonas (4%) and Ochrobactrum (4%). This seems typical.

The most prominent bacterial species were R. picketti, B. cepacia, S. maltophia and Ochrobactrum anthropic.

We need to aggressive monitor and periodic sanitize our water system to keep the bacterial count down and discourage the formation of biofilms. The monitoring I would recommend would be the use of biofluroscent viable particle monitoring supplements by R2A agar-based monitoring.

A19: I'll add that USP 60 gives a method for testing water for Bcc if that's what you'd like to test. It's not quantitative, just presence/absence. Ensuring your process is robust and your product is free of objectionable organisms is the goal.

Hold Time Requirement for bioburden samples

Background: we are a low bioburden operation that manufactures a Drug Substance. Fill and finish is done at a CMO. We test bioburden on intermediates all along the process, 14 sample points in fact. (This plan is a bit overly conservative.)

For the testing, we send to a contract lab. While the majority of samples are tested within 24 hours, occasionally samples are tested outside of this 24 hour window due to scheduling, holidays, etc. I've been tasked with writing a position paper to have at the ready if we are challenged on having an occasional sample tested outside of the 24 hour window, with the concern being these samples wouldn't be representative. Questions:

1. For our argument, is it enough to point to the conservative sampling plan and say that if an isolated sample exceeds the 24 hours, it doesn't matter in the grand scheme, which includes the pre-filtration bioburden the CMO performs? (i.e. "If sample X was tested at 48 hours and showed no bioburden, and all other samples before and after in the process tested within 24 hours showed the same, there is no concern sample X was not representative.")

1. If #2 isn't enough, is this a possible scientific argument: the factors that cause the phenomena of bioburden reduction in liquid samples due to sample storage aren't present in our intermediate samples as they are in purified water and WFI? (e.g. hypotonic VS isotonic environment)

(Is USP <1231> the only place this recommendation is found? Can anyone provide the underlying studies that support this recommendation? The USP doesn't and my Google searches have proved frustrating.)

1. Based on #2, is bioburden reduction in the sample, producing a false pass, really the concern, as much as proliferation would be, causing a false failure? If the main concern is the latter, that would easily be caught given the other sample results all along the process showing acceptable bioburden, correct? And despite that, am I thinking correctly that proliferation is a business risk primarily, in that you could throw away a perfectly good batch? (I realize there's risk of drug not being available to patients, but I want to leave that aside.).

A1: IMO…there’s not much difference [microbiologically] between 24 hours vs. 48 hours, i.e., if the samples are stored properly. There is nothing magical about these time points; only that we’ve been conditioned to follow them, rather than challenging their absoluteness. Instead of backing yourself into a

corner, be proactive and give yourself some “wiggle-room” to accommodate circumstances which delay testing (e.g., weekend/holiday periods).

You mentioned the word “proliferation” a number of times. I suggest you send the drug substance out for Water Activity (Aw) testing to determine if the material has the capability to support growth/proliferation. Couple that with a historical review of your bioburden data for the conservative sampling plan that you mentioned.

I did this novel work during the 90s (contributing to the creation of USP <1112>) and it explained a lot as to how some raw materials and finished products examined for microbiological content always came back with no microbial load. The combination of Aw and MLTs' (now <61>/<62>) results, scientifically-justified a reduction in testing. This approach may work for your organization.

More detailed information can be found in the articles below (along with plenty of references):

Friedel R.R. “The Application of Water Activity (aw) Measurement to the Microbiological Attributes Testing of Raw Materials Used in the Manufacture of Non-Sterile Pharmaceutical Products,” Pharmacopeial Forum, Vol. 25, No. 5, pp. 8974-8981, 1999.

Friedel R.R. and A.M. Cundell. “The Application of Water Activity Measurement to the Microbiological Attributes Testing of Nonsterile Over-the-Counter Drug Products,” Pharmacopeial Forum, Vol. 24, No. 2, pp. 6087-6090, 1998.

A2: Scott my first reaction is to ask why are you manufacturing a biological drug substance at a site that has no microbiology lab.

A3: We have one, but at the time of filing we were using a contract lab.

PCT Failure

Please help me with the investigation of failure for Preservative Challenge testing of Powder for suspension.
Upon constituting with water, PCT testing was performed for this product using USP Standard 5 organisms.
Although all lots with lower preservative concentrations passed, there was a failure observed for Pseudomonas aeruginosa in 2 lots with full strength preservative (parabens).

1. Upon check with the analyst, all steps were followed.
2. All media and equipment were ok
3. Sample solution incubated at 20-25C looks ok
4. Analyst is trained and has executed at least 40-50 PCT testing.

What should be checked for verifying that there is no lab related issues.
Is there any special considerations for Powder for suspension PCT test?
What are the chances that this is a true failure?

A1: You might have a chemistry issue. One of the possible explanations as to why the product failed challenge testing is that the parabens are not very soluble in water if you are reconstituting a powder formulation that contains parabens. When formulating with parabens, you have to either heat the water phase of the formulation to dissolve the parabens or use a solvent to dissolve the parabens in order to get the parabens into the water phase of the formulation in order for it to kill microorganisms.

As to why the lots with a lower concentration of parabens passed and the full strength preservative system did not, what did you use as a filler in the lots with a lower preservative concentration? If you did not use a filler, it could also be that the lower concentration of the parabens was slightly more soluble in the reconstituted water than the full strength concentration that showed better antimicrobial activity.

A2: The literature informs us that powders have a history of inactivating preservatives:
McCarthy, T.J. (1969) “The Influence of Insoluble Powders on Preservatives in Solution,” J. Mond. Pharm., Vol. 4, No. 12, pp. 321-329.
Horn, N.R. et al. (1971) “Interaction Between Preservatives and Suspension Systems,” Amer. Perf. Cosmet., Vol. 86, pp. 37-40.
Myburgh, J.A., McCarthy, T.J. (1980) “Inactivation of Preservatives in the Presence of Particulate Solids,” Pharm. Weekblad Sci. Ed., Vol. 2, pp. 137-142.
Myburgh, J.A. and T.J.McCarthy (1980) “The Influence of Suspending Agents on Preservative Activity in Aqueous Solid/Liquid Dispersions,” Pharm. Weekblad Sci. Ed., Vol. 2, pp. 143-148.

That being said, it seems a bit odd that the lower concentrations passed, while the higher ones failed. Can you supply us with the actual data for the time points tested?

PET USP <51>

I would like your expert opinion on USP <51> "log reduction calculation"

As per USP <51> the following statement has been given for the log reduction calculation.

Using the calculated concentrations of cfu/mL present at the start of the test, calculate the change in log10 values of the concentration of cfu/mL for each microorganism at the applicable test intervals, and express the changes in concentration in terms of log reductions. The log reduction is defined as the difference between the log10 unit value of the starting concentration of cfu/mL in the suspension and the log10 unit value of cfu/mL of the survivors at that time point.

As per above statement for log reduction initial concentration of cfu/mL present at the start of the test needs to be calculated .

My query is whether it is calculated from the organism inoculated in diluent/Neutralizer (without product) or organism inoculated in test sample, which one should be consider for the initial cfu/mL for the log reduction calculation.

Please help to understand the above statement. Which should be considered as a starting concentration cfu/mL either from positive control which is run along with test sample or initial concentration cfu/ml of the test sample.

A1: The initial inoculum count must be taken from the organism inoculated in diluent/Neutralizer (without product). Even a short time in the product may cause loss in viability.

MB quality of water after softener

We get water (Aqua fontis) from a supplier (testing is done), it goes into the purification system and we test it after softener, final product (Purified water) is also tested on a declared frequency.

Highest MB count in the system is obtained from water after softener.

We test it the same way we test Aqua fontis, and we have the same action limits for them (20CFU/ml for inc. 22°C and 100CFU/ml for inc. 37°C).

Where can I find guidence for MB limits for Aqua fontis and for water after softener?

A1: I’m assuming that when you obtain aqua fontis from a supplier is that you are getting spring or well water as the source water for your water purification system. My Latin is a little rusty.

Because this source of water needs to be in compliance with drinking or potable water standards, it has to be in compliance with the National Primary Drinking Water Regulations (NPDWR) (40 CFR 141) issued by the U.S. Environmental Protection Agency (EPA) or the drinking water regulations of the European Union, Japan, or the WHO.

Water softeners are used to remove Calcium, Magnesium and other cations that can cause water hardness. In water softeners, microorganisms can increase and may serve as a contamination source in downstream water system components. Biofilms are known to form in these units.

It is highly unlikely that a water sample taken directly after a water softener unit in the generation portion of a purified water system will be in compliance with the enumeration levels of purified water. Because high levels of microorganisms will be obtained after a water softener, I would recommend the usage of a pour plate procedure instead of a membrane filtration procedure for enumeration. I have never seen anyone establish enumeration levels of water samples taken immediately after multimedia filters, carbon filters, water softeners, and ion-exchange columns. However, I have seen the establishment of enumeration levels of water after ultra-violet lights and micron cartilage filters where it will be expected that microbial levels will be low.

A2: Water from supplier is spring water and it is in compliance with drinking water standards (<100 CFU/ml).

I have found this suggestion for limits for MB quality of water after softener (WHO PQ Workshop, Abu Dhabi, 2010, Tony Gould):

Sampling location	CFU/ml		
	Target	Alert	Action
Raw water	200	300	500
Post multimedia filter	100	300	500
Post softener	100	300	500
Post activated carbon filter	50	300	500
Feed to RO	20	200	500
RO permeate	10	50	100
Points of use	1	10	100

They do not give limits for water after CEDI module.

Can we just change the limit to 500 CFU/ml (we have specification for Aqua fontis, so it is specification limit)?
What other actions would we need to make if we were to change the limit?

visual inspection of sterility media canisters

How do you perform visual inspection of media canisters at interim and end of incubation reads of the sterility test: per USP 71

"At intervals during the incubation period and at its conclusion, *examine the media for macroscopic evidence of microbial growth*. If the material being tested renders the medium turbid so that the presence or absence of microbial growth cannot be *readily determined by visual examination, *14 days..."

Separately for particulates per USP 1790, 790 and inspection of injections under a light box and black & white background is required. Media inspection after a media fill is also performed under the light box due to an observation. This has been extended to the sterility media which may be overkill.

Is there justification to utilise a light box and black and white backgrounds for the examination of sterility media canisters during interim and end of incubation reads?

I would argue that the intent is different of the two chapters and that we are looking for "macroscopic evidence of microbial growth" in the Sterility test. Further, the sterility verification is based on clear visual turbidity within 5 days for the compendial organisms.

How do other members feel? And what is the practice for sterility test media examination? Have you been challenged to inspect media under a light box and with blanck and white backgrounds specifically?

A1: A good question. The compendial sterility test does not detail the background, lighting, and visual competency of the inspector, presumably because it is not critical to the test method.

The microbiologist will have experience in detecting signs of microbial growth in the media by virtue of conducting growth promotion tests and investigating the occasional sterility failure.

As the product may create turbidity, instrumentation that does not rely on the appearance of growth, i.e., respiration with CO2 sensors or headspace analysis, ATP production, etc are being more widely implemented to reduce subjectivity.

A2: If a facility is using the light box would it be seen as a "lesser standard" to go back to observe visually for microbial growth without the black and white background. I still feel that the USP 71 does not have a specific expectation so there is not quality "reduction" in removing the backgrounds. However if this was in the context of detecting turbidity related to product it may be seen as reducing detection/detectability.

One of our product lines displays sporadic non microbial turbidity. It is tested at several sites but only one site uses this background and only that sites sees this incident occurring. Removal of a perceived "enhanced detection" puts us at a difficult place with QA as fear of a loss of detection increases risk of a true failure being ignored. We are discussing other technologies to remove analyst detection but meanwhile we need a work around.

Any shows of hands where using a black and white background is normal is appreciated. Would like to discuss what led you down that path.

A3: My experience has been that lighting to examine the canisters need only be adequate. White and black backgrounds are not required.

A4: Using specialized lighting with a white or black background is used for visual inspection of parenteral products not sterility testing.

Here the challenges are different. A typical product may be 1 ml in a 2 ml tubular or moulded vial with the most common defect foreign particulates. Also an inspector looks at hundreds of vials over a shift.

With sterility tests you examine a limited number of canisters containing 100 mL of medium for more obvious signs of microbial growth.

A5: Yes we do interim observation of the sterility test canister.

Light source (Black and White Background) help in better visualisation of growth, cloudiness/turbidity in the test canisters, that is the only reason for using this.

Interesting FDA Warning Letter Comments

I get a weekly email notice for FDA's latest Warning Letters and they are often very educational. Their website is https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/compliance-actions-and-activities/warning-letters?utm_medium=email&utm_source=govdelivery

This one warning letter highlights two important areas of concern:

The first point is a lack of finished product testing (low-hanging fruit for an Investigator).

The second point deals with an item that many manufacturers have had issues with. Specifically the point details a lack in-coming API and component testing and the companies reliance on a manufacturers C of A. This seems to be a common occurrence is some manufacturers in-coming QC process - simply approving a C of A. It seems to happen when the organization uses overseas manufacturers of API's and raw materials and components and does not have the ability to audit those manufacturers.

The following is taken directly from the warning letter:

"2. Your firm failed to conduct at least one test to verify the identity of each component of a drug product. Your firm also failed to validate and establish the reliability of your component supplier's test analyses at appropriate intervals (21 CFR 211.84(d)(1) and (2)).

Based on the records and information you provided, you have not demonstrated that you are testing incoming lots of the active pharmaceutical ingredient (API) used to manufacture your OTC drug products to determine their identity. In addition, your firm released an API for use in drug manufacturing based on a component supplier's analysis report, although you had not established the reliability of the analysis through appropriate validation.

For example, in response to our initial 704(a)(4) requests for information on identity testing for each lot of component, you did not provide evidence of such testing. In the subsequent t-con held on January 19, 2021, you stated that you do not perform identity testing for each lot of incoming (b)(4) but rely only on vendor Certificates of Analysis (CoA). In the same t-con we requested documents containing the testing performed to qualify the (b)(4) supplier. In response to this request, you provided documents on February 1, 2021, which included Control Forms, Analytical Method, and a third-party Analysis Report for (b)(4). The documents provided are not enough evidence to demonstrate vendor qualification and establish the reliability of your (b)(4) API supplier.

You can rely on vendor CoA for quality attributes, provided you conduct at least one test to verify the identity of each component lot before use in the drug product manufacturing. Additionally, you should have established reliability of supplier's analyses through adequate initial validation and subsequent verification of supplier's test results at appropriate intervals."

This comment is telling " You can rely on vendor CoA for quality attributes, provided you conduct at least one test to verify the identity of each component lot before use in the drug product manufacturing."

A1: it is a 21 CFR 211.84 requirement to conduct an identification test for drug substances and pharmaceutical ingredients. For drug substances many companies assay the material. The vendor CofA is reviewed and risk-based testing may be conducted to verify that other critical quality attributes meet specification.

Another issue around identification testing is whether to extend this testing to every drum of an incoming lot or test a representative sample. With the advent of NIR spectroscopy each drum could be checked on receipt or during the batching process in manufacture.

USP water - sterile water for irrigation

I'm in the UK and looking to purchase purified water that complies with USP (TOC, Conductivity and TVC). I can find PW to EP but that has different conductivity limits.

A supplier can provide sterile water for irrigation (USP), however they have a statement saying "this solution is not isotonic and is hemolytic". what does this mean and is it an issue?

Is this water able to be used in place of USP PW and of a higher quality? I thought that it was WFI so meets/exceeds PW parameters but it is slightly less quality relating to particles so cant be used in parental preparations, but that is not my concern.

A1: I have searched to buy PW that meets USP specs, it is challenging.

SWFIrrigation is not a replacement for PW. The PW specs for conductivity and TOC are much lower the cond/TOC specs for SWFIrr. This is because the container can adversely impact these chemical measurements.
Yes, the sterile water is better than PW for meeting microbial requirements - since it is sterile. But not the chemical measurements.

A2: PW for USP Is equivalent to Highly pw for EP.

A3: This small difference is causing us a big issue.
Whilst trying to solve this supply issue I'm trying to use the available info in the pharmocopoeias to provide and document the information to show suitability.

Additional scenarios for discussion for you/anyone else please.
If we are defining USP PW for product manufacture, however i am only able to source sterile PW (USP) what are your thoughts on being able to use this, as the USP allows for sterile packaged PW to have a higher conductivity, e.g. 5us/cm for containers larger than 10ml. I believe that this product must be derived from PW and meet the requirements for the bulk water lower conductivity limit of 1.3us/cm. Im hoping that the fact that we must purchase rather than produce on site gives us the different limit to use for sterile PW when purchased but it is still classed as PW and can be used.

The conductivity will end up being completely different as soon as you start product manufacture and adding other excipients and API. Do we just need to show that whatever PW we use (be that bulk produced in site-1.3us/cm or purchased sterile PW-5us/cm) is used and meets the limit for that type defined in USP?
this was my train of thought with the sterile WFI (USP) as its teh same conductivity limit for sterile PW so i wanted to argue interchangeable.

A4: It is tough to answer. If your SOPs call for use PW, then you could be at risk using SPW. I follow your logic, PW is used to make SPW. The probably is that the SPW container does adversely (usually) impact the cond and TOC, thus the higher limits.
A literal reading of your SOP would say you are not conforming to your own procedures - if you replace PW with SPW. Many persons do think SPW must be better than PW. It is, microbiologically. Not chemically.

Is it possible the SPW meets PW requirements for cond and TOC? Keep in mind the following - if you test the SPW like it is PW, the stage 2 limit is 2.1 uS/cm, not 1.3. You got a little more headroom.

Frankly, a regulator may accept SPW as equal or superior to PW. But from pharmacopoeial point of view, I literal reading of specs does not work in your favor - in my opinion.

USP water and sterile water - (Conductivity)

USP PW and WFI (@25 degrees C) has conductivity of 1.3us/cm for stage 1 and 2.1us/cm at stage 2 with stage 3 pH.
however it states for sterile packaged alternatives there is a limit of 5us/cm

if you intend to manufacture with PW an define that in IND, but need to purchase PW in containers as you can not produce at your site and purchase sterile PW or WFI is that ok to allow the higher conductivity value or do you need to assess that you are accepting the higher limit against the defined lower limit?

As an additional, Pharm. Eur. packaged sterilized water has lots of additional tests, I'm assuming to assess packaging contamination, but there is no similar in USP. Any ideas why it is a lot stricter?

A1: Yes, conductivity precludes the wet chemistry tests.

A2: i was trying to review the pharma chapters again to respond but keep getting caught up on the differences.

I can see that the the USP sterile PW limit for >10ml containers is 5us/cm but starting water must comply with the bulk limit.
In the EP i cant find sterile PW only PW in containers and it doesn't specify sterile. Is there a definition of sterile PW as i think i may have confused myself.
The PW in containers has a 5.1us/cm at 25C along with all the additional chemistry testing.

Although one is sterile whilst other is not they both have similar conductivity but EP has the additional tests. Would the lower initial value for USP before it was put in a container still stand for precluding the additional tests that EP define, as the conductivity limit they apply is the same for the bulk and packaged.

A3: You are correct, there is no EP monograph for Sterilized PW. In fact, their PW in Containers is just that, PW that is in a container with micro limit of 100cfu/mL and no sterility test (obviously).
Yet there is a EP Sterile WFI monograph.

While USP does have a Sterile PW monograph, its used is called out only 4 times in the entire list of USP tests.

While I argue that the added chem tests (for EP SPW) are not necessary and redundant, it is what the book says. HOWEVER, I have it on very good authority that the "committee on water" (I am not sure that is the correct name) in EP is STRONGLY considering following USP to remove all/most the chem tests. What is the logic? You passed all chem tests by meeting the cond test for the PW. Is there any reason to think you would fail a CO_2 or ammonia or chloride or calcium... test just by filling a plastic bag? I" do not know about deletion of the OxSub test in lieu of a TOC test like USP.

Aliquot impact on CFU calculations (i.e. LOD, DF)

I have some questions about dilution factor (DF), limit of detection (LOD), and final result calculations based on differing aliquot sizes when testing product. We don't routinely test anything beyond 1 mL after dilution, so a product neutralization effort raised a question of larger aliquots in testing. This kind of caught me off guard. The calculation we are using is such:

(Average of counts/aliquot) x dilution factor

Does the amount of aliquot affect the dilution factor or LOD in any way?

Example 1: Plating 1 mL of a 1:10 dilution (10 g product in 90 mL diluent), we interpret no growth as <10 CFU/mL. The DF and LOD both are 10 as I understand. 0.1 grams of product ends up plated.
Example 2: Plating 1 mL of a 1:100 dilution (10 g product in 990 mL diluent) we interpret no growth as <100 CFU/mL. The DF and LOD both are 100 as I understand. 0.01 grams product ends up plated.
Example 3: Plating 10 mL of a 1:100 dilution (10 g product in 990 mL diluent).

1. 0.1 g product ends up plated, correct?
2. If 0.1 g plated, has the DF changed to a 1:10 since the physical amount of material being tested is the same as example 1 or remain at 1:100?
3. Has the LOD changed to 10 or at 100 (no growth interpreted as < 10 or < 100 CFU/mL)?

A1: Your detection limit is affected by the aliquot plated in addition to the dilution. You will need to do the math to determine how much sample material is being represented on the plate.

In your examples:

1. 1mL of 1:10 is <10 CFU/mL limit (0.1g material represented)
2. 1mL of 1:100 is <100 CFU/mL limit (0.01g material represented)
3. 10mL of 1:100 is <10 CFU/mL limit (0.1g material represented)

Growth of aspergillus in SCDM broth

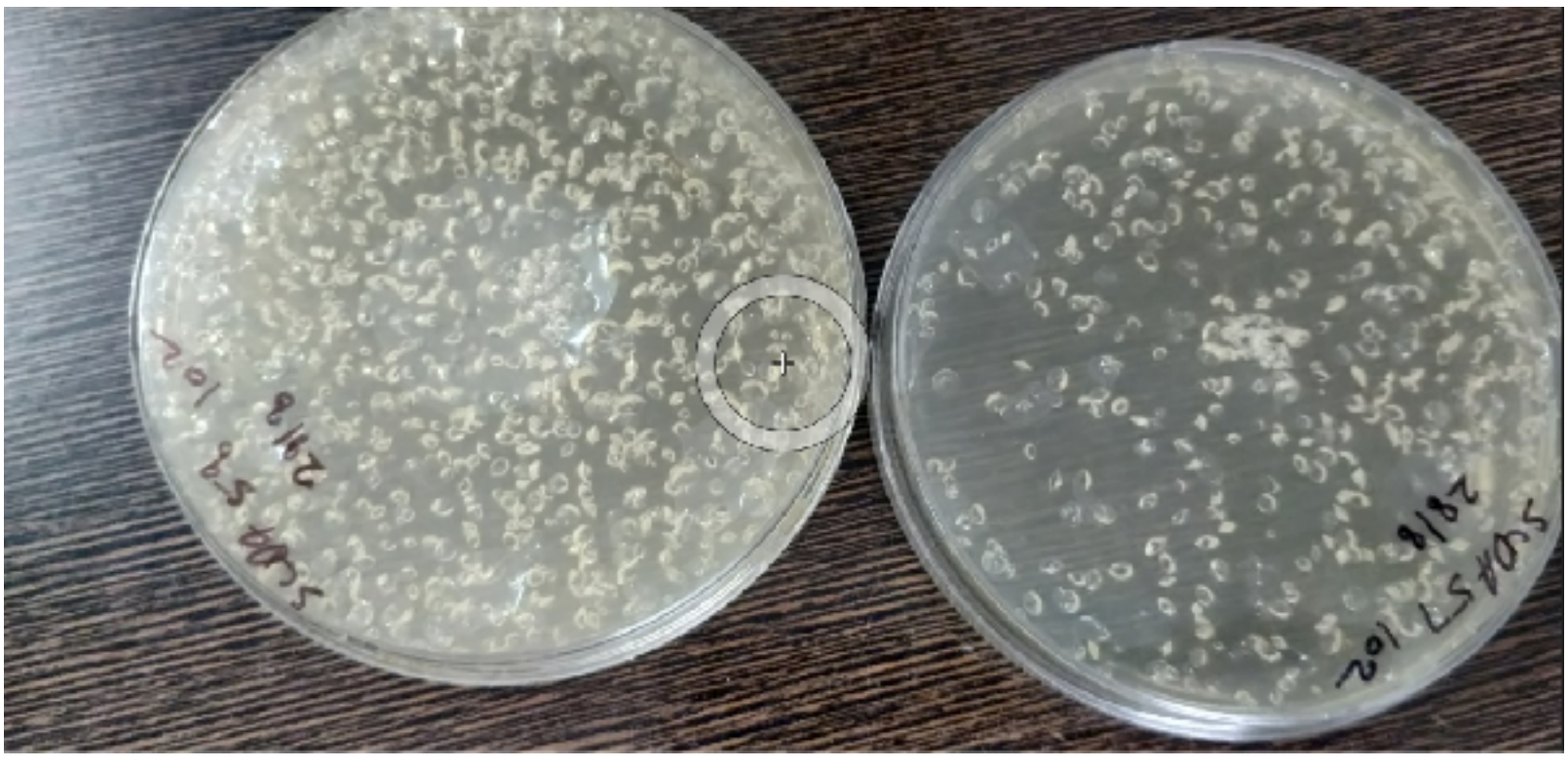
We are using Aspergillus bioball for gpt of scdm. But we are getting growth
after 5 days.
Pls let me what could be the problem and how can resolve this issue.

A1: It could be that the pH of SCDM might be somewhat inhibiting the growth of Aspergillus spores. You have to remember that the pH of SDA and PDA are around a pH of 5.6. I believe that the pH of SCDM is around 7.1.

Unusual bubble formation

Please give your suggestions and comments on this plate. It's a tablet sample
for Microbial enumeration test.

However no colony is observed, but bubble formation is there, suggesting some
growth. But no visible CFU are there.
What should I report in this case. And what phenomena is this basically.



A1: Noted the bubbles. Looked like a few yeast, maybe.

What ingredients are in the tablet that could release CO2? Do you have a carbonate in the ingredient deck? Also, what pH is your media? Is your media acidic?

A2: Interesting case but could you please provide more background information so that we can provide more suggestions? Says, what exact tablet is it? Is it something like effervescent tablet? Are you using SCDA? How long and what temperature did you incubated the plates? I doubt if the gas was produced by microorganism but a result of a chemical reaction.

A3: First you can enrich the agar from the plates in scdm to conclude whether the particles can grow or not in liquid media and based on that you can conclude for that particular batch.

It might be due to cross reaction between media and product. Need to observed plates after product inoculation and before incubation. Can get some clue by verification of ingredients of products tested and it's properties.

A4: After looking at the screen shot, there are no microbial colonies present in each of the Petri dishes. Without knowing the composition of the tablets, I suspect that the bubbles are from carbon dioxide generation from a tablet ingredient that came into contact with water from either the microbial count diluent or the microbial growth agar. In addition, do these bubbles appear immediately or after a period of time for incubation. Effervescence from a tablet ingredient usually occurs immediately when it comes into contact with water.

A5: Did you come across this issue during qualification testing of the method?

A6: These were three batches of temozolamide capsules. Only two showed this kind of formation.

Thanks [name redacted] […. Carbonate specifically I will check. These were capsule of temozolamide. And media was SCDA only so ph was 7 , nothing acidic as such.

Thanks [name redacted].....but these were not effervescent tables. Yes the media was SCDA. This gas formation was observed on 2nd day at 34degree, might be chemical reaction because no special cfu was observed.

Thanks [name redacted]... Yes by that way I can check if some growth is there or not or just a chemical reaction.

A7: It is my understanding that temozolomide will break down to an active metabolite plus carbon dioxide at neutral and alkaline pH values. I suspect that the bubbles in the plate count agar are a result of carbon dioxide being generated from the breakdown of temozolomide at the pH of SCDAM.

A8: [name redacted] post confirms the value of a microbiologist understanding the chemistry of a material.

Endotoxin Calculation

i need a suggestion regarding endotoxin calculation.
One powder for injection has drug substance 100 mg and 3000 mg excipient.
it use as a infusion and infusion rate is 3 mg per kg per hour.
K= 5 Eu per kg per hour so
EL is 5/3 = 1.67 Eu/ mg
in this case, we should consider excipient or not. because excipient is very high.
Please suggest.

A1: The excipient must be considered as well as the solution for injection (WFI?).

Your question is not entirely clear. Is this final product testing? All things that could contribute to the final endotoxin load need to be considered. Are you testing the API and the excipient already combined? Is this in process testing where they have not yet been combined? What volume of diluent will the final product be suspended in?

USP <85> Bacterial Endotoxin

I need some guidance regarding chapter <85> USP Bacterial Endotoxins.

I understand that some raw materials used in injections have established parameters to perform this assay (i.e. Sodium Chloride) and even tell you the concentration to work with. But how can I establish specifications to perform the assay in those raw materials used in parenteral preparations that don't have one? (i.e. calcium chloride used in Ringer, or diclofenac).

Also, I don't understand the calculus when the limits are expressed as mg/mL when LAL Reagent is in EU/mL

A1: When you mention raw materials, I am assuming you are talking about Finished Product used in the preparation of IV solutions. These products come with a certificate of analysis, including bacterial endotoxin limit and analysis result. A number of products and some raw materials have a USP monograph, stating the BET limit, but not all of them. Regardless, the BET limit also needs to be calculated, based on worst-case dosing information provided in the product insert, using the USP <85> K/M; where K is the threshold human pyrogenic dose of endotoxin per Kg of body weight (5.0 USP -EU/Kg/hr.) and M is equal to the maximum recommended human dose (mg) of product per Kg of body weight (70 Kg for an average adult weight). When the product is to be injected at frequent intervals, or infused continuously, M is the maximum total dose administered in a single hour period. Then limits are compared - The lowest limit is used as worst-case.

Once a BET limit for BET is established for Finished product, the active ingredient plus any other excipient is taken into account to assign a BET limit to each component. The principle is that a combination of material in the formulation should not exceed the Finished Product limit. I do believe this same principle should be applied when diluting a product into IV solution.

Regarding your last question - The limits are not expressed as mg/mL (this is product concentration) - Limits are most of the time expressed as EU/mg (some exceptions are EU/Unit, EU/Meq, etc). This is an example on how a limit is calculated (assuming a maximum daily dose of 300 mg/day or 12.5 mg/kr.):
Finish product (FP) Limit = K/M = 5.0 EU/Kg/ k. / (300mg / 70 Kg x 1day/24 hrs.)
= 5.0 EU/Kg/ hr. / (300mg/70 Kg/24 hrs.) = 5.0 / 0.17857 mg/Kg/hr. = 28.0 EU/mg (Finished product limit).

Anhydrous citric acid

Can anyone help by sharing the procedure for BET test by gel clot method for Anhydrous citric acid?

Micro specs for patches

Please share your opinions on how do you interpret microbial specifications for patches as per EP 5.1.4 Do we express TAMC & TYMC per gram /ml or per patch?

A1: Transdermal specifications for TAMC and TYMC are CFU per patch.

A2: I believe that in Table 5.1.4-1 of this section 5.1.4 that the microbial count for patches is expressed as CFU/g or ml. I think that this is more appropriate to report the results in CFU/g or ml because patches can be in different sizes and composed of different materials. I believe that there is no standardization in the composition of patches.

setting atypical and out of trend limits

is there any guidance on how to set trend limits or levels for ascertaining if a count is atypical or out of trend?

my specific situation is that we test non-sterile products, raw materials and componentry and these routinely have zero cfu (per g/ml/unit). However when we obtain a sporadic low level count e.g 1-5cfu we now make a judgement about performing an initial lab investigation or not but don't have any formal guidance to refer to. when we obtain higher counts e.g. 20cfu against a limit of 200cfu it feels more appropriate to perform an initial investigation.

either way the counts are all well within specification but im trying to create some guidance that I can proceduralise.

TAMC/ TYMC Sample Compositing

We currently composite beginning, middle, and end batch samples for final micro product testing.

When is it acceptable to test a composite for the batch samples? If we composite and one sample is contaminated, but the other is not, how do you assess the risk involved?

A1: The success of the sampling plan will be influenced by the number of organisms in the material and the distribution within the material. A statistical-based plan may be based on a Poisson distribution. To determine the distribution and number of organisms per unit weight of material, especially with low numbers, taking multiple samples and testing them separately would work best. Taking multiple samples and making a well-mixed composite sample may be better for a presence/absence test but could underestimate the number of organisms in the bulk material

With pharmaceutical ingredients and drug products we typically assume they are well mixed, so materials with moderate to high counts a single 10 g sample will be suitable.

We microbiologists need advice from our statistical colleagues.

A2: It is generally acceptable to composite samples for micro tests, such as what you have described. This can be done for quantitative or qualitative analyses.

In your scenario, if you composite samples, and the result is positive for contamination, it is not possible to know which of the individual samples are contaminated, as only a single composited sample is tested. As such, you implicate the entire batch as being contaminated. Even if you tested the B, M and E samples separately, you may not be able to isolate the extent of the contamination For example, if the middle sample were contaminated, you don’t know how far upstream or downstream that contamination could go. Without such information, it would be difficult to justify releasing any of the batch.

If you recently experienced a contamination event, then testing individual containers from different parts of the manufacturing/packaging process may be performed, to try to isolate the root cause, assuming you are able to pull filled containers from different phases of your process. But this is only for investigative purposes only.

MLT METHOD DEVELOPMENT FOR NON STERILE PRODUCTS

Here i am having a query about the non sterile finished product method development. My query is: if we did not get any recovery by any method (as stated in USP-NF 2021 CHAPTER NO 61) what could be the inference we supposed to give to the respective product, and what is the alternative?

A1: For non-sterile products that contain antimicrobial preservative systems, recovery may be possible using neutralizers, dilution or using a membrane filtration method instead of a pour plate. Keep in mind the sensitivity of the method with respect to the microbial specification. If the active ingredient has inherent

antimicrobial activity, and you cannot recover the challenge organisms in the method suitability test, the organisms most likely would not survive in the material so you are justified for not running the test.

A good source of information is USP <1227> Validation of microbial recovery from pharmacopeial articles.

A2: While performing method suitability test you can increase the number of rinse in membrane filtration method.(NMT 5 Times).

Reduced testing of non-sterile products

Do we have to test every batch of manufactured product or can a justification for reduced testing be performed?
e.g. based on trend history, assessment of manufacturing process, ingredients or other aspects.

Any guidance references or experience appreciated.

A1: There are circumstances where you do not have to test every batch of non-sterile product. See ICH Q6A and decision tree #8.

A2: You can follow the guidance of ICHQ6 Decision Tree 8 to justify your 'skip lot testing'.

Submission of your report to the regulatory body will lead to an acceptance or decline of your proposals.

So no you don't have to test every batch of every product, but yes you will have to justify your decision.

A3: Please refer to the ICH Q6A decision tree.

A4: When they state "demonstrate the inherent antimicrobial activity of the drug product" in decision tree #8 what exactly does that mean? Would an MIC and/or PET test be enough to demonstrate these characteristics of the product using the compendia organisms?

A5: Based on ICH Q6 Decision Tree #8 and the GMP regulations you can forgo microbial testing of low-risk non-sterile drug products when justified to the FDA. For instance, you may not include a microbial specification in the regulatory submission.

FDA GMPs: The FDA CGMP regulations 21 CFR 211.113 Control of microbiological contamination states: a) Appropriate written procedures, designed to prevent objectionable organisms in drug products not required to be sterile, shall be established and followed.

Furthermore, 21 CFR 211.165 Testing and release for distribution (b) states: There shall be appropriate laboratory testing, as necessary, of each batch of drug product required to be free of objectionable microorganisms.

A6: The MIC would be suitable for antibiotics and antifungals agents and the PET for other products.

For example, it makes no sense to run microbial tests on an anti-tumor drug.

A7: I do agree with you that MIC testing would be more acceptable for antibiotics and antifungals. However, I have a question for you.

When you are indicating PET test data for a product formulation can be used to justify skip lot testing, what preservative challenge test acceptance criteria are you using?. For example, are you suggesting that USP AET challenge acceptance criteria such as a 2-log reduction of bacteria and no log reduction for fungi at 14-days is acceptable as a justification to conduct skip lot testing?

In reality, I would think a 5-log reduction in all challenge test organisms at 2-days for a product formulation would be a better justification to support skip lot testing than using USP AET challenge test acceptance criteria. By having a more stringent challenge test acceptance criteria, it shows that the product is hostile to the survivability of contaminating microorganisms. I'm interested in hearing your perspective in regards to this question.

A8: I'm sure [name redacted] will reply but I wanted to underline the very essence of 'skip lot'.

It should not be undertaken without experience, subject knowledge and product understanding.

In reverse order:-
"By having a more stringent challenge test acceptance criteria, it shows that the product is hostile to the survivability of contaminating microorganisms".
[Only to a finite point e.g. Aw 0.1 does not reduce the activity of microbial proliferation any more than 0.3]

"In reality, I would think a 5-log reduction in all challenge test organisms at 2-days for a product formulation would be a better justification to support skip lot testing than using USP AET challenge test acceptance criteria".
[Yes I imagine it would be, and a 12 log reduction even better, but is it necessary]

"For example, are you suggesting that USP AET challenge acceptance criteria such as a 2-log reduction of bacteria and no log reduction for fungi at 14-days is acceptable as a justification to conduct skip lot testing"?
[Yes and No, the presence of other growth hurdles will support that justification]

"When you are indicating PET test data for a product formulation can be used to justify skip lot testing, what preservative challenge test acceptance criteria are you using"?.
[Product PET acceptability may change by type and use but its use to support 'skip lot' has to be applicable to the same mode of action and be considered alongside other hurdles to growth]

I wouldn't want the general message here to come over in a manner that the application of skip lot testing is a simple task to apply in a slap dash fashion.

Reduction in testing cannot and should not occur unless the overall risk of any process can be documented and justified to not increase.

A9: In ICH QC6, the justifications for conducting skip lot testing is vague. Before implementing skip lot testing, you need to look at many other things besides the results in conducting QC microbial testing and preservative challenge test data of a formulation. For example, I would include looking at water activity of the formulation, cleaning and sanitization practices of the manufacturing facility, susceptible raw material to microbial contamination being tested for microbial content, facility environmental microbial test results, and whether the purified water system is a hot or ozone circulating system. I would be very hesitant to institute skip lot testing for a formulation of the purified water system is an ambient temperature circulating system.

I fully understand as the main reason for instituting skip lot testing as a cost saving measure and to have a quicker release of a batch.
However, I think that you need a better justification than PET and QC microbial count test results for instituting this policy. I just question whether the current PET test acceptance criteria is sufficient justification because it does not identify hostile formulations from those that are adequately preserved from my viewpoint .

A10: I believe we should be testing water systems more frequently especially if they are maintained at ambient temperature, taking advantage of the biofluorescent viable particle monitoring systems that are being more commonly used.

I am not a big advocate of skip lot testing for microbiology preferring a risk-based approach and mitigating any risk of microbial contamination and eliminating all testing. I find the tendency towards annual testing a single lot laughable. A fig leaf.

A11: In early product development we typically use both USP and Ph. Eur time intervals and log reduction requirements.

For FDA product submission you need to demonstrate you meet the USP <51> requirements.

Resource documents and publications to follow

I'm looking for people to share what web sites, mailing lists, resource documents ect you subscribe to or prompt yourself to review on a frequent basis to keep up to date on current thinking, issues and information applicable to the pharma industry.

e.g looking at FDA 483's, checking USP-NF notices.

A1: Try www.fdanews.com/form483.

Classified area

Can anyone please guide me? I am going to use a single AHU for both classified and unclassified areas(Microbiology testing lab (Solid oral dosages)). Terminal HEPA filtration used in classified and normal filtration used in unclassified areas. If it is ok what type of circulation can we use?

A1: It is not ok. you must arrange 2nd AHU. it is main difference between classified and unclassified area that is HEPA filter and separate unit.(AHU) when you use single one for classified and unclassified area then may early chock/leak the HEPA filter and you can not judge it.

Purified water sample

As per USP <1231> chapter water samples for Microbiology analysis shall be performed within 2 hrs otherwise store the samples at 2 to 8°C for 24 hrs. Is this for each sample (if samples 10 or more) i.e first sample or after completion of sampling activity.

A1: Yes it shall start from 1st sample. if we wait till all samples sampling the bioload in the 1st sample will proliferate and gives false positive results.

A2: If your sampling activity itself takes few hours- the first sample(s) taken will be at risk of getting exposed to high or low transient temperatures for greater than 2 hours. In such case a controlled temperature portable storage basket should be carried to ensure that the previous samples taken do not get exposed to high or low temperatures for extended period of time while entire sampling is complete. A state of control is what you need to show to ensure that the microbial integrity of your samples was not compromised- date/time samples taken, sampling person's initial, temperature monitoring of the portable storage container, date/time the samples were tested, tester's initials, etc. would show that you have a good state of control.

A3: After Water Sampling storage should be done for individual samples. Means first sample should be stored within 2 hrs of sampling.

A4: KINDLY PROVIDE COMMERCIAL PRODUCTION PROCESS OF BIO FETLIZERS

A5: As a worst case, from first sample, 2 hrs timeline shall be followed.

A6: Thanks for your information regarding water samples, is there any reference or guideline for water sample collection , transportation and analysis timelines, During water system validation more than 30 or 40 samples are required to analyze at a time required more than 2 hrs. If more samples are there in validation time can we divide the total samples into set 1 and set2 like that and perform the analysis. If we perform like this all the set point sample need to cover 2 to 4 week validation time or any single set samples cover minimum 2 week time can be considered. For this is there any reference document or guideline available.

If samples are not analyzed within 2 hrs we have to store 2-8 °c , is there equipment anyone using to store 2-8°c and carry the water samples other than ice packs.Please provide equipment details.

FDA Guidance Document on Microbiological Quality Considerations in Non-sterile Drug Manufacturing Guidance for Industry

Today, the FDA just published a draft guidance document called "Microbiological Quality Considerations in Non-sterile Drug ManufacturingGuidance for Industry."

I believe the comment period on this proposed document is open until
December 29, 2021.

A copy of this document can be obtained at:
https://www.fda.gov/regulatory-information/search-fda-guidance-documents/microbiological-quality-considerations-non-sterile-drug-manufacturing

Rapid Sterility via PCR

Is anyone doing rapid sterility via PCR for short shelf life products? Such as using Sartorius' Microsart ATMP Bacteria and Microsart ATMP Fungi kits? Looking for information.

70% Denatured ethanol and DET repeat or not

We have had DET performed, by a contract company, following ISO standards, on a disinfectant (e.g. 70%DE) using different various coupons/surfaces that we assessed as being required.

if we want to move to a different supplier of the same type of disinfectant do we need to repeat the full testing again? or is it a case that we have performed it on a specific type and it doesn't matter who manufactures it?

My thoughts as it is a pretty standard alcohol solution then they are all similar. It is not like it is a specific formulation of a disinfectant that the supplier has designed.

A1: if anyone has any expert opinion or previous experience on this topic and my situation that would be greatly appreciated.

My thoughts are that it could be justified but we have only undertaken this exercise once and relied on contract lab for testing and now i need to assess if it can be leveraged as acceptable for introducing an alternative but same type of disinfectant.

A2: I believe you can justify this as a like-for-like change and forgo disinfectant efficacy studies.

A3: I agree with [name redacted]. Just be sure the application (ie spray and wipe) is kept consistent with what was done in the study.

Shellac

Do you perform microbial test on Shellac raw material?
We need to perform an investigation but we have issue about the raw material's solubility.
If you perform the test, can you suggest us how to dissolute the Shellac, please?

A1: Please provide additional information about your shellac. Is your shellac in a resin form of flakes, alcohol solution, or an alkali emulsion? Shellac flakes are soluble in alkaline solutions such as ammonia, Sodium borate, Sodium carbonate and Sodium hydroxide and also in various organic solvents such as ethyl alcohol and acetone. In general, aqueous based shellac dispersions and coatings are susceptible to microbial contamination.

Release testing of microbiological media

We are currently testing each shipment of media for growth promotion and sterility. Sometimes we will get 3 or 4 shipments of the same lot. Is it acceptable to release media based on passing results of a previous shipment of the same lot, providing that the vendor has a validated shipping procedure?

A1: Growth Promotion should be done per shipment in addition to per lot to determine if actual shipping conditions were adequate to support the media received, regardless of whether a vendor validated a shipping procedure at one point in time. If a truck gets stuck for a day (or a few) in hot temps, will that effect the media's ability to support growth? That's proven / those instances are answered by performing the test per shipment and per lot.

A2: USP 1117 talks about testing media and how shipping conditions can negatively affect media. So even if it's the same lot...each shipment needs to be tested because the shipping conditions could have changed. Interestingly, has anybody justified NOT doing GP testing when a temperature control device is used and shows the product being in spec the entire shipment? I haven't heard of it but would love to know if it's happening.

A3: I have not seen much discussion in the pharmaceutical realm, but have seen it in the clinical realm.

Clinical labs have a standard (CLSI M22-A3) where the retesting of the listed commercially prepared microbiological culture media is unnecessary for those media that are of proven reliability for recovery of clinically significant microorganisms. This standard was based on the fact that in the US, prepared media is categorized as medical devices and quality control data surveys conducted by the College of American Pathologists (CAP). As medical devices, it is incumbent upon the manufacturers of prepared media to ensure that it's protected from alteration during "customary conditions" of distribution.

I'm actually quite surprised that there hasn't been an alignment between pharma and clinical in this regard.

A4: I have been aware of the divergence of opinion between clinical and pharmaceutical microbiologists on the need to conduct growth promotion testing of general microbiological growth media for many years.

I agree with our clinical colleagues and your statement. Reliability of this media has been proven by CAP proficiency testing but GPT in the pharmaceutical industry is entrenched as a cGMP requirement.

A5: "Entrenched" is the quintessential description.

A6: I do understand that shipping conditions will affect the media. However, with a validated shipping procedure combined with continuous monitoring to confirm that the media remains within the specified conditions throughout the process, wouldn't that be sufficient to justify the GP testing per lot rather than per shipment? Of course, if the monitoring shows that the conditions stray from the specifications, that would trigger a need to perform a GP test or possibly an outright rejection of the shipment.

A7: If you are able to document shipments, conditions, growth promotion results and failure rates to create a scientific justification for GP on a lot to lot basis, not every shipment, and get the regulatory bodies to agree.....people on this PMFList will buy you endless beers. And I'm not being glib...I think it's a good idea with current monitoring technologies and room-temperature stable media to challenge this “entrenchment”.

A8: It is mandatory to perform GPT for each lot, but if you are having validated shipping procedure i.e. maintain the temp. Condition and retrieve the data for objective evidence you can go for reduce test i.e GPT with in-house flora (1 or 2) instead of using all standard cultures.

Candida albicans ATCC 10231 Inoculum Recovery

I need some guidance regarding the recovery of Candida albicans ATCC 10231.

We are currently initiating GPT for our first media (brand new lab): TSA and Sabouraud Dextrose Agar and had some inconsistencies with our inoculum of Candida albicans.

The test was performed as follows:

1. Test strain prepared in Sabouraud Dextrose Agar, incubated at 20°–25° 2–3 days.
2. Suspension made with Phosphate buffer solution pH 7,2 and adjusted at 0,5 McFarland using a calibrated Densitometer.
3. From this 0,5 McF suspension (teoric 1,5x10^8 UFC/mL) serial dilutions were made, up to Dil 6 (teoric 1,5x10^2 UFC/mL).
4. Dil 3, Dil 4, Dil 5 and Dil 6 were inoculated in both media (0,1 mL inoculum) and incubated at the same time: TSA at 30°C-35°C and Sab at 20°C-25°C.
5. The recovery obtained in Sab Dextrose Agar was as follow:

- Dil 3 (teoric 1,5x10^5 UFC/mL, inoculum 1,5x10^4 UFC mL) mean plate count: 183 UFC.
- Dil 4 (teoric 1,5x10^4 UFC/mL, inoculum 1,5x10^3 UFC/mL) mean plate count 13 UFC.
- Dil 5 (teoric 1,5x10^3 UFC/mL, inoculum 1,5x10^2 UFC mL) and Dil 6 (teoric 1,5x10^2 UFC/mL, inoculum 15 UFC/mL) mean plate count <1 UFC.

6. The recovery obtained in TSA was as follow:

- Dil 3 (teoric 1,5x10^5 UFC/mL, inoculum 1,5x10^4 UFC/mL) mean plate count: 241 UFC.
- Dil 4 (teoric 1,5x10^4 UFC/mL, inoculum 1,5x10^3 UFC/mL) mean plate count 26 UFC.
- Dil 5 (teoric 1,5x10^3 UFC/mL, inoculum 1,5x10^2 UFC/mL) and Dil 6 (teoric 1,5x10^2 UFC/mL, inoculum 15 UFC/mL) mean plate count <1 UFC.

NOTE: We understand the McF scale is an approximation not an exact count, still the recovery rate is minimum. The same procedure was made with the other strains used with both media and this is the only one with issues.

Is there a reason why our recovery was way less than estimated? Has anyone had issues with this strain? Please advice.

A1: I've always used a higher McFarland for this strain. Try 2 McFarland. I have no idea why this strain doesn't behave like the others, but microbes pretty much do what they want.

A2: Your work is perfect.

The reason is that C albicans is a microorganism which, when suspended, gives a greater turbidity than bacteria. Mac farland refers, as a turbidity match, to bacteria and not yeasts. So you worked correctly and the results are in line with the turbidity obtained for yeast cells.

A3: McFarland turbidity Standards were originally developed for use in predicting the concentration of bacterial cells such as E. coli or K pneumoniae. I think what is happening in your case is the yeast cells, being much larger than bacterial cells, measure at a higher turbidity with a lower cell count. You may need to develop a correction factor to continue to use the standards with C. albicans.

A4: It looks to me your counts are right on target where Candida should be at 0.5 McF but you have overestimated the starting titer and that is why you recovered less colonies than expected. Your theoretical titer of 10^8 seems to be based on bacteria instead of yeast. Since individual yeast cells are larger than bacteria cells, a suspension of yeast will have less cells per mL at the same McF value.

My own personal rule is that Candida is typically 2 logs less than bacteria. For example, an overnight tube of broth will saturate at around 10^9 cfu/mL for most bacteria, but will be only 10^7 cfu/mL for Candida and appear to have similar turbidity. At 0.50 McF, Candida should be around 1.5 × 10^6 cfu/mL.

A5: Candida is a unicellular yeast, and much larger in size as opposed to bacteria, against which the McFarland Turbidity units are calculated. Hence you will end up less CFU when plated on agar. In order to verify the original suspension you made and matched against the 0.5 McFarland Unit, you could count the yeast cells in the suspension using a Haemaocytometer /counting chamber and match the colonies recovered in GPT against the counts.

A6: I agree with [name redacted] comment about the yeast concentration and McFarland turbidity. Instead of using a correction factor, I would suggest using a higher McFarland concentration to prepare a C albicans suspension for inoculum preparation. Try a 1.8 to 2.2 McFarland concentration to see if you get the approximate concentration of C. albicans conc. for a PET inoculum.

A7: I will be performing again the test using a higher McFarland concentration, I was so worried with the strain itself that the detail about cell sizes truly slipped my mind.

I do really appreciate you took time to enlighten me, sometimes a fresh set of eyes is needed to solve the problem.

A8: I agree with both [names redacted] here. Definitely try using a higher McFarland standard as I recall using a standard of 2 when I was performing PET with C. albicans for precisely the reasons explained below.

A9: Why are we using McFarland Standards when spectrophotometric methods are available?

A10: We're entrenched.

A11: We use the same strain for disinfectant efficacy testing (EN 1650).

When preparing the test suspension with saline, we use 3,0 McFarland concentration. This gives us a concentration around 2 x 10^7 cfu/mL.

I think, it is better for you to find the right McFarland concentration for each microorganism that you are going to use. While trying to verify densitometer's calibration when we first had it, different calibration kits gave different results, even though they claimed to have the same McF. concentration. It might be because some use latex particles and others use $BaSO_4$. It is not possible to know what was used by the manufacturer.

I know it seems a minor detail, but it was crucial for our analyses.

A12: Unfortunately we do not have a spectrophotometer in premises yet, there has been a few delays with our provider… but we do have a densitometer and an urge to start working on GPT.

A13: It's possible that a digital densitometer is being used rather than the old-school method of comparing turbidity against physical barium sulfate McFarland standards and stripe card. A digital densitometer reading in McF units is essentially doing the same thing as a spectrophotometer.

A14: Never mind, it's never had it's 3 month maintenance or calibration.

I'll do both. Note, they are 3M not monthly. Is that ok?

A15: C. albicans, being a yeast is much larger than bacteria. The number of organisms in e.g. a 0.5 Mcfarland for a yeast will contain significantly less organisms than a bacteria.of the same standard.

A16: Similar to the query beings answered I have similar question. Recently we have conducted the study in our laboratory for microbial recovery using McFarland values of different microbial culture but did not get the countable results in any serial dilution. Refer attached screenshot for results. Need the guidance to get accurate results.

A17: First thoughts are:
1. Check that the dilutions were done correctly
2. Check that the proper dilutions were plated
3. How long were the dilutions held, and at what temperatures, prior to plating?
4. How long were the plates incubated?

Leveraging Media Fills on a multi-product line

I am assessing a sterile filling line thats about to add a similar product to be manufactured on that line. The manufacturer's desire is to leverage the media fills from one product to make the same claims about the aseptic processing of the other. I noticed that the final sterilizing filters for both products are different (in brand, at least - they are still .22 micron sterilizing filters). Would the different filter manufacturers / different filters be reason alone to NOT leverage media fills between products, and to instead perform the required media fills separately per each product? What other factors should be considered here when making this comparison / assessment?

A1: What are the container, closure and speed differences?

A2: My understanding is that a media fill is largely used to validate the aseptic filling operation and sterile filtration validation with a B. diminuta challenges addressing the sterile filtration process and are product related.

A3: A lot to consider. Line speed. Fill volume. ALL interventions associated with the product fill(s). Container size. Any possible filter differences and interactions with the product. Number of personnel present. Etc.

A4: I'm currently investigating the differences in these filters.

A5: The use of a different sterilizing filter for different product usually doesn't prevent the leveraging of media fill data. The aseptic processing simulation challenges all processes and product contact surfaces downstream of the sterilizing filter. Filter validation studies (PDA TR-26 is my favorite reference) will help evaluate the suitability of the filter with the product and processing conditions. If all of the manufacturing/ media fill processes downstream of the sterilizing filter are the same for both products, then leveraging media fill data may be appropriate.

A6: Brand filter is not as important as the materials of construction.

A7: With regard to the filters, there are very specific tests that are run, including microbial retention studies using B. diminuta. They are usually outside of the scope of most pharma labs, but the filter manufacturers can generally help in performing these studies. In regard to leveraging the media fills, it may be possible, but you have to make sure that you do a very well defined, clear risk assessment. Don't do what I have called a GPS one, where you know you where you want to get and you risk assess it so that you get there.

Environmental monitoring - Solid oral dosage forms

Solid oral dosage forms are manufactured in grade D area.
We monitor it monthly and we have over 11 000 samples yearly.

It takes a whole team to manage this - we do all the media preparation, sampling (swab method) and testing/reading the results, trending etc.

Superiors demand we reduce the testing according to historical data. I have some ideas on how to do it (analyse perhaps last two years of results, once again decide whether the sampling spot is critical etc.).

What is the best way to do this and comply with GMP?
Do you have any advice/guideline on how to make EM monitoring easier for ourselves?

A1: You are definitely thinking along the correct lines. Any changes to an EM program should involve a risk analysis and be based on historical data when possible. It sounds like you have a couple of years' worth of historical data. You can begin by reviewing this data and determining if there are some sampling locations that may no longer need to be sampled. Or perhaps some locations could be sampled less frequently. You can also perform a risk analysis on each sampling location to determine if the criticality for sampling has changed.

Be sure that whatever changes you decide upon are documented, justified, and defendable to potential questions from auditors.

A2: Actually there is no regulation that requires solid oral dosage form to be manufactured in a Grade D environment.

Evidence of this belief in the September 2021 Draft Guidance For Industry Microbiological Quality Considerations in Non-sterile Drug Manufacturing that make no reference to this requirement.

I would refer you to the best source of guidance.o USP <1115> Bioburden Control in Non-sterile Drug Substance and Drug Product Manufacturing

I believe you are wasting your company's resources. by monitoring this aggressively.

A3: Please refer to USP 1115 which guides to prepare a risk assessment to fix an EM program. A whole set of parameters to be considered in the risk assessment are specified. The results of previous trends are always helpful to assess a meaningful data of the EM and to reduce the frequency and (or) sampling sites.

In general I have seen companies following a frequency ranging from once in a month to once quarterly.

A4: You can minimise the sampling location based on historical data, Use risk based approach for selection of sample location.

Refer USP <1115> for Microbial assessment of nonsterile product manufacturing environment.

If you are performing Active as well as passive sampling, then you can select only Active air sampling method.

Swab sampling also minimize, rationalize with cleaning validation of equipment in place.

A5: I agree with the comments already made by others on this topic. It does seem like a huge amount of work. However, if you are unsure about how to perform the risk assessments mentioned in some of the responses, I urge you to consult with any of the consultants who regularly post on this site. The cost of a good consultant, I am sure, can overcome all of your annual costs to perform this testing.

A6: You can reduce your sampling frequency to quarterly once instead of monthly once You can rotate all the sampling point according to your sampling plan. No. Of sampling location depends upon the area, operation so you could decide the sampling location based on that's. If your facility is in state of control having low bioburden in the area, you can reduce the sampling location based on the historical data

Non viable particle testing

For non-viable particle monitoring in aseptic manufacturing areas, what size particles are sampled for in ISO 7 and ISO 8 rooms in the US? USP chapter <1116> and FDA Aseptic guidance only specify a limit for 0.5µm . What is the US expectation, sample and monitor only the 0.5µm size, or sample 5.0µm as well per Annex 1?

What limits are applied for non-viable particle monitoring in ISO 7 and ISO 8 rooms? Are different limits used based on the status of the room? For example, applying ISO 8 limits in an ISO 7 room during dynamic conditions? This is what is stated in Annex 1 but haven't been able to locate anything this specific in US guidance. What is the expectation for the US on the status of the room and the limits applied? What is industry doing in the US?

For non-viable particle count excursions in ISO 7 and ISO 8 manufacturing areas, what kind of corrective actions have been employed? What typed of things are investigated to determine product impact?

A1: It is typical for both 0.5um and 5.0um data to be saved, and where Risk dictates alert and action limits are applied. A useful guide for the process is the PDA Technical Report 13, Fundamentals of an Environmental Monitoring Program, 2016 and this will give a review of practices etc to best meet the needs of your facility. The PDA also has several volumes of Environmental Monitoring Handbook, edited by Jeanne Moldenhauer that would also makes for useful reading.

Brazil Pharmacopeia Water testing

I am trying to find out if the Brazil Pharmacopeia specifies media type to perform purified/WFI water testing e.g. R2A, mHPC, or leaves selection open to the QC lab?

A1: It seems Anvisa references WHO..that references EP and USP...so....back to square 1.

A2: In the topic below you can see the 02 medias applied, unfortunately the 6ª edition version is available only in Portuguese.

https://www.gov.br/anvisa/pt-br/english/pharmacopeia

Farmacopeia Brasileira, 6a edição page449

5.5.3.6 ENSAIOS MICROBIOLÓGICOS DA ÁGUA PARA USO

FARMACÊUTICO (5.5.3.6 Microbiological tests for water to pharmaceutical purposes)

* Ágar para contagem em placa (plate count agar - PCA)
* Ágar R2A

A3: The Ph Eur has an official microbial method whereas the USP in <1231> cites the AWWA/APHA Water Standards.

I believe that the Eur. Ph method in R2A agar incubated at 30-35 degree C for 48-72 hours.

Evaluating Paperboard Packaging

I am looking for information for evaluating paper packaging (thin sheet paper or paperboard) that will come in contact with low moisture product like soap bar. There are methods that test the fungi by placing a defined piece of the paperboard on an agar plate and inoculating the surface of the paperboard to see if the mold grows. Is placing the paper on an agar plate realistic to what the paperboard would experience in real life in warehouse or shipping, since it is sitting on a puddle of water? Has any one evaluated in a humidity chamber etc or have any other experience with these products and risks?

A1: To evaluate the resistance of paperboard for soap bars to mold grow, I would suggest that you use ASTM 2020 in which separate inoculums of 3 mold species are used to inoculate samples of the cardboard that is used to either wrap or contain a soap bar. Many times this cardboard has been treated with an anti-mold agent to prevent the grow of mold.

A2: This method is withdrawn

ASTM D2020-92(2003)Standard Test Methods for Mildew (Fungus) Resistance of Paper and Paperboard (Withdrawn 2009)

A3: Another approach is using physical attributes like water content and water activity to determine the susceptibility of the paperboard for fungal growth. Water content around 16-18% and water activities >0.75 would encourage fungal growth.

A4: You are correct that ASTM D2020 Standard Test Methods for Mildew Resistance of Paper and Paperboard had been withdrawn in December 2009. However, it should be noted that the standard had been withdrawn due to insufficient committee interest in reviewing and revising the standard. I have found no other standard test method that can be used in place of it. In addition, it should be noted that this particular withdrawn standard can still be purchased from the ASTM website. I see that a lot of contract microbiology testing laboratories are still offering the usage of this standard for evaluating mildew resistance of paper and cardboard.

If people do not want to use ASTM D2020 because it had been withdrawn, it may be possible to modify the following standard test methods to determine the mildew resistance of paper and cardboard:

ASTM G1-Standard Practice for Determining Resistance of Synthetic Polymeric Materials to Fungi

AATCC 30-111 Test Methods for Antifungal; Activity, Assessment on Textile Materials: Mildew and Rot Resistance of Textile Materials.

When I had conducted work to determine the mildew resistance of soap bar wrappers and cartons, I had used ASTM D2020 on numerous occasions and found it to be a satisfactory test method. I do not understand why it was withdrawn by the ASTM committee.

A5: I agree with you. Even Though the method is withdrawn for reasons other than technical, I do use them for testing purposes. I just wanted to mention it , just incase they want to quote and claim passing tests oer a Standard ASTM Method.

For testing fungal defacement on any fungal prone surfaces,, there are many approaches as you have listed. One just needs to place the substrate of interest in a high humidity (>90% RH) chamber, or a box and incubate at ~25 to 28C temp, and the fungus will grow. ASTM G21 is also a method one can adopt, or

ASTM C1338, Or a new test although meant for coated surface, ASTM D 7855 can easily be used for the surface defacement studies on uncoated materials.

A6: I believe that the formation of mildew on paper or cardboard that is used to wrap soap bars is a little more complicated than just performing a water content or water activity level of the paper or cardboard wrapping. There are 4 different processing methods for making soap bars. If a melt and pour or a hot manufacturing process is used to form the soap bars, they are usually packaged immediately after formation. As the bars cool inside either a paper wrapping or cardboard box to room temperature, water condensation occurs from the atmosphere inside the box which allows the growth of mold spores on either the paper wrap or cardboard box. From my perspective, it has nothing to do with the water activity or water content of the wrapping or cardboard box.

A7: Aw of soap wrap (paper or board) in use is dynamic both with small direct uptake after application (bars may be often cured/cooled before wrapping) and more so as the soap subsequently exudes/ evaporates moisture - esp. if shrink wrapped into bundles. Bars are often cured before wrappingCorrugated cases are also at risk - esp. in shrink wrapped context. In my experience, risk with true "soap" is greater than surfactant-based "soap."Aw of paper wrap or board as packaging material is irrelevant - except for their resident fungal spore content.

gram negative rods in non-sterile products

With regards to Gram negative organisms that are specified as required to be absent <1111>, are we actually allowed to isolate other general Gram negative rods and release a product? im assuming yes as otherwise all would be identified as being listed, however it doesn't seem right.

In addition to isolating from the product, what about componentry material that we have assigned the same spec as the product. So, still not on the specification but again doesn't feel right.
if we do need to consider other not specific negatives, do we have to take into account probability/risk. e.g 1 cfu of a Gram negative rod isolated from a can. what is the likelihood of this making it into the can being tested and being isolated from the amount of material tested.

A1: Cosmetic specs typically include - no detected Gram negative bacteria flor both product and ingredient. One need merely look at those listed in relevant enforcement reports to understand this spec.

A2: My opinion is also that general gram negatives are an issue in the higher risk non-sterile products. However, why don't the pharmacopoeias just state 'absence of Gram negative bacteria' as a blanket statement rather than detailing a few.

This is then interpreted as you only need these ones and if you do isolate a ram negative you can review and deem it acceptable.

Endotoxin Hold Times for Purified Water

I have a question regarding the holding time of purified water samples used for endotoxin testing.

We are currently using the Nexgen PTS & cartridges for our Endotoxin testing. The testing we do for our water on site isn't an issue since we test it the same day it is sampled, but in the next few months we will be picking up the endotoxin testing from one of our sister sites.

As the shipping site cannot do a T0 test, and I have an ~24 hour transport time, I am not certain how to proceed. I considered using a sample from our site, in order to have a T0 then ship it, and have it returned. That is however just a reflection of the 48hr transport time. That might resolve the issue of the initial transport, but we also need to consider we may not be able to run all the samples in one day. That leaves me looking at a window of 24hrs transport plus 48hrs holding all at 2-8°C. I could of course test that

sample (or samples) multiple times over the course of a few days but I am not sure it would be sufficently representative.

Essentially, does anyone have experience with this, or would be able to offer any advice?

A1: I believe you need to perform hold time study by spiking endotoxin in the same sample container if you are using Nalgene depyrogenated bottle as plastic has the tendency to adsorb endotoxin and it will affect your endotoxin recovery.
Someone can question that you are reporting false negative results.

In addition, if you are using PTS & cartridges for Endotoxin testing, you can still do BET on the sampling site and no need to transport samples.
Per USP 1231, the requirement is to perform BET within 12 hours of sampling.

A2: I understood purified water, USP does not have a bacterial endotoxin specification. Is the water designated as low-endotoxin, purified water?

A3: We recently just transferred water testing for our sister sites as well. To validate the hold times, our sister site would send about 4 sets of the same water sampling ports to both our site and the contract lab that was currently testing their waters. We tested the first set at approximately 24 hours, the second at 48, the third at 72, etc. The contract lab did the same thing with their waters. We compared the results and calculated the percent difference between them. This also provided justification that at the 96 hour hold, the results were the same as the 24 hour hold allowing us to utilize a long hold time. Hope this helps.

Turbidity method USP 81

We are trying to develop the turbidity method for one of our drug products. We are using Candida tropicalis ATCC 13803. As per our API vendor's method, the difference of %T between extreme doses of standard should be greater than 40. (Growth condition for organisms is 29-31C for 4-5 hours at 250 rpm).

We ran multiple trials (different dilutions and incubation time range) but ended up getting not more than 25%T difference between extreme doses of standard. See the table below.

USP Reference Standard concertation (ug/ml)

%T at 530 nm
0.02 26
0.04 34
0.06 40
0.08 44
0.10 48

What should we try to increase the %T difference between 0.02 and 0.1 ug/ml of drug concentration?

A1: in turbidimetric methods we use time is also very important factor.
Active substance uses microorganism (in this case C.tropicalis) as a substrate, so the individual %T should be greater with longer time it has to react with substrate.

I can think of few other factors:
1. %T of the suspension of microorganism (I guess you make suspension of C.tropicalis) that you start with;
2. Dilution (as you already tried) of suspension/higher conc. of product/standard

Other idea is to try and get API vendor to share some more information.
They should also benefit of you getting the method right.

EMPQ concurrent with Media Fill

Based on your experience/knowledge, is it acceptable to perform an initial EMPQ of part of a room and part of an isolator during a media fill? Wouldn't this be considered worst case as it isn't simulated dynamic conditions but actual dynamic conditions.

Otherwise, does the EMPQ need to be completed prior to the media fill?

A1: It is quite common to perform the EMPQ concurrently with the media fill.

Disinfectant Dwell or Contact Time

Hi folks: Does anyone know of a specific reference for maintaining a wet surface as the definition of Dwell/ Contact time? I know it is required, but could not find a reference.

Next question: Has anyone qualified the use of one temperature for a media fill- e.g., 28-30 and if so, what were the criteria for acceptance of that?

A1: ASTM E2111 − 12 is a good reference for dwell/contact time with sanitants. I believe the single temp media fill has been validated but I can't give you any concrete case study. Here's a PDA paper that talks about the validation of that concept.

https://pubmed.ncbi.nlm.nih.gov/27593691/

A2: There are various approaches that were used to support use of a single incubation temperature. Some companies have referenced data from published literature on rapid sterility testing. There are companies that showed which temperatures were "worst case" for microbial growth. In those studies, the single temperature used most often was either 32 deg C or 30 -35 deg C. There has also been work performed with environmental monitoring and incubation with different or single temperatures.

Often, my clients run a protocol evaluating the recovery of organisms at different temperatures ranges between 20 and 35 deg C to see what works best (or equivalent).

Most of the regulatory reviewers have looked for a good rationale to support what was is conducted.

A3: PDA TR 70 Fundamentals of Cleaning and Disinfection Programs for Aseptic Manufacturing Facilities makes specific reference to a wet contact time for disinfecting. Here is their definition of Contact Time, "The minimum amount of time that a sanitizer, disinfectant, or sporicide must be left in complete (wet) contact with the surface to be treated in order to be effective." Wet contact time is referred to elsewhere in the document as well.

Regarding the media fill incubation question, the FDA Guidance on Aseptic Processing states, "Incubation temperature should be suitable for recovery of bioburden and environmental isolates and should at no time be outside the range of 20-35 C." Within this statement are the acceptance criteria, i.e., the ability to recover bioburden and environmental isolates. In-other-words, the incubation temperature chosen and qualified must be supported by product and EM data regarding the types of isolates that are recovered at the facility. 30 C +/- 2 C may be the most appropriate as it allows recovery of most mesophilic organisms. Lower temps may impair the recovery of Gram-positive cocci per USP <1116>, while higher temperatures, e.g., 32.5 +/- 2.5 C may impair recovery of some environmental molds and psychrophiles (personal experience).

A4: <1072> defines the contact time as the time after application with the expected log reduction. The lab studies are simulations of the facility disinfection where the surface is not constantly kept wet but dries out after application.

USP <61> reduced quantity clarification

USP <61> provides justification to reduce the quantity of sample tested from the 10 gram minimum. I had a couple questions interpreting this section:

<61> states that for active substances specifically (API), the quantity can be reduced to 1% of the total batch, when the batch size is small (less than 1000 mL or 1000g). Is that 1000 mL or 1000 g referring to the amount of active substance in the final bulk formulation, or total weight of the finished product batch?

<61> also states that the number of finished product units can be reduced to two when the batch size is less than 200. Can the rationale for reducing active substance also be applied to finished product? If small capsules are being manufactured with a batch size of greater than 200, but the total weight of the final product is less than 1000 g, could a justification be made to test 1% of the total batch weight instead of 10 grams? It seems odd to me that USP <61> instructs you to test a minimum weight, but then allows you to reduce finished product quantity based on “units”.

A1: The topic of the Interpretation of USP <61> section "Testing of Products", Amount used for the Test, also cropped up recently in a thread initiated by Reuven Baum.
The wording in the section of Paragraph 2 regarding reduced testing for product is particularly convoluted.

The interpretation , or at least my understanding of it, initially centres on whether the material is an API or Product (Intermediate/ Formulated)
and thereafter in the case of formulated product, on the active content and the defined dosage type.

For General Testing (Starting Materials, APIs. Bulk, Intermediate Finished Product etc)
10g / 10ml or a composite of 10 specified dosage units (patches /aerosols) is the normal standard.

Reduction for APIs
For API batches of < 1000g / 1000ml a single test preparation of 1% of the batch is acceptable but only for API.

Reduction for Product in Defined Dosage Units (< 1mg Active)
For products in defined dosage units (capsules, tables, vials, Injection etc) with an active content of <1mg, test quantity reduction can be achieved by compositing 10 dosage units. While the wording seems to suggest that other product preparations with an active content <1mg may be considered, the caveat is in the last line "In this case the amount to be tested is not less than the amount present in 10 dosage units, 10g or 10ml. of product". Effectively if the product is not presented in defined dosage units, the 10g or 10 ml standard quantity applies.

The 1k /IL batch with a single test involving 1% of batch is not applicable to your scenario. Its only relevant to API.

If your capsule is a defined FP dosage unit (how is it described in your manufacturing Authorisation and CTD /Dossier etc) and has an active content of < 1mg, Paragraph 2 would apply, and it would seem OK to composite 10 capsules per test preparation.
Similarly for Reuven's situation with a lyophilized sterile, if the actual active concentration in the dose was < 1mg, it might be possible to composite 10 dosage units, reducing the actual test quantity to 1g rather than 10g.

If the relevant batch size of defined dosage units contains less than 200 or 100 units, the number of test preparations can be reduced to 2 or 1 respectively.
I'm assuming that this means that otherwise, 3 tests are the minimum standard requirement and that GMP considerations will demand that appropriate statistical sample plans are applied to standard / large batches etc..

All things being equal reduced test requirements will have to be justified, approved and documented in the CTD/ Dossier etc and so there are checks and balances.
These are pragmatic approaches taken to balance quality testing needs with practical Production / R &D considerations, particularly with respect to small volume, highly concentrated and high-cost Active Ingredients.

Hope this helps and always interested to hear others interpretation where different or more nuanced.

Use of poly bags and HDPE drum in sterile manufacture

When using polybags or HDPE plastic containers (non-sterile) when weighing non-sterile raw materials to be used in a sterile filtered or bulk sterilized product, is it industry standard to test each lot of the poly bags or containers for microbial quantity prior to use?

If each lot is not tested, is it typical to be tested when qualifying the bags or containers for use only and not with each batch?

Max Gowning Time

Are there any white papers or documents supporting maximum gowning time? (i.e. maximum time that associates can be gowned in the cleanroom before they need to come out and change).

A1: I do not think there are any white papers to support one time or another. You will need to include whatever your allowance is with your media fill. You may want to go a bit longer in the MF as inevitably during normal processes, someone will stay in too long.

Escherichia coli ATCC 8739

I am currently assisting a college thesis, and need to compare a current result obtained with *E. coli* strain. To test storage conditions of lab prepared MacConkey Agar, we used 3 separated lots of Agar, each one prepared and sterilized individually and at 4 time spans (T0: 0 days, T1: 15 days, T2: 30 days and T3: 45 days).

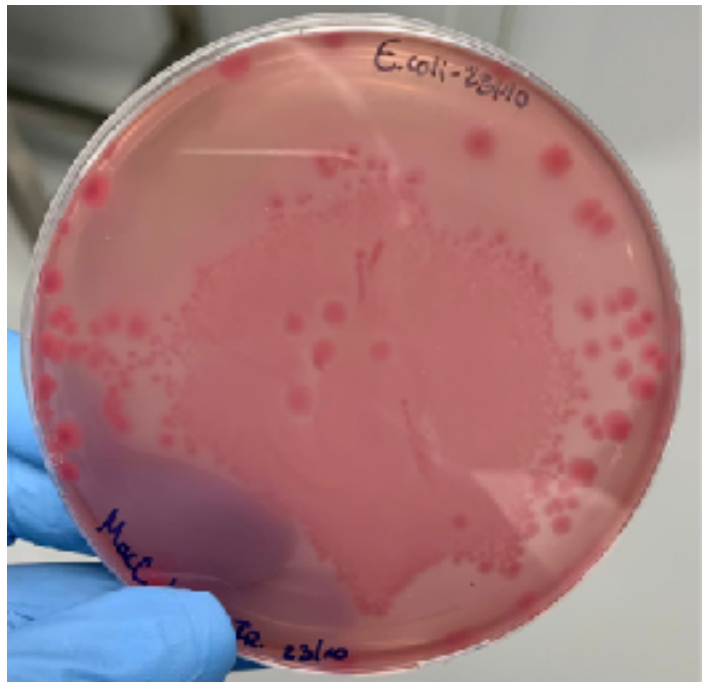
However, our growth shows like the attached photo.

We saw this growth in all 3 lots and up to Time 2, but not at the same time. At T0 was only Lot 3, at T1 were Lot 1 and Lot 2, at T2 were Lote 2 and 3. (Inoculum: 100 uL of 6x102 UFC)

The GPT performed previously didn't have any similar issue.

Glass pearls were added, suspension vortexed, strain freshly prepared (4th passage), spreaders changed... but still same results.

Has anyone ever had similar issues with this specific strain?



A1: How did you store your plates after preparation , were they refrigerated before drying for all time periods? It looks like the agar plates were not fully dried and you could get these smearing growth if you spread them on a wet plate.

A2: You do not disclose the ATCC strain of E. coli . Was it the type strain for the species or the strain recommended in USP <62>.

What could be the source of the variability? Lot to lot variability in the formulation, the medium preparation and sterilization, the medium storage conditions, the E. coli preparation, the storage of the inocula. the inoculation of the plates, i.e., a pour or spread plate inoculation, the incubation conditions, etc.

The Difco manual recommends incubation at 33-37 degree C for 40-48 hours with the colonies color of pink to red due to lactose fermentation with bile precipitation.

A3: The subject line states that it is E. coli ATCC 8739 strain.

Since she mentions , the media were prepared and stored,, I assume they were already poured plates.. Also 100uL was used and they mention spreader, so these were spread plated.

A4: I suspect the probable cause is overheating during the sterilization process. Are you leaving the media in the autoclave after the slow exhaust cycle diminishing the growth-promotion capability of the medium?

A5: Just to clarify some questions.

1. Storage Conditions that were tested: Poured plates: 90mm x 15mm. Refrigerated at 2°C - 8°C. Schott Bottle 500 mL: At room temperature 25°C ± 5°C.
2. Inoculum: 4th passage. Tested both prepared prior to inoculating and prepared 3 days prior. Both gave similar results.
3. Media sterilization: 121°C 15 min, removed from the autoclave as the cycle finished.
4. GPT: Performed for this specific agar without any trouble, with good recovery.

A6: I would concur with [name redacted]i that this is likely a spreader issue due to wet plates.
This can happen if plate surfaces and the interface between the edge of the plate are overly wet due to condensate presence when removed from the fridge.
Generally insufficient cooling of pates prior to refrigeration is the most common culprit for presence of excess condensate.
If plates are not fully solidified and / or the agar is still relatively warm (e.g. > 10oC) when placed in the fridge - condensation will occur to various degrees primarily depending on initial agar temperature as well

as subsequent cooling rate (so plate position within a plate stack or stack positioning will also influence the level of condensate). That could also explain the variable lot performance.

It's important to follow/ establish effective SOPs suitable for your operation that consider control steps for;
1: Tempering molten media prior to plate pouring,
2: Sufficient equilibration time to allow plates reach room temperature before refrigeration and
3: Routinely Drying Plates (using suitably controlled aseptic practice) at 30oC for a defined time) on removal from refrigeration.
4: Ensuring plate surfaces are not visibly wet at the post-inoculation / pre-incubation step.
Needless to say, the suitability of all steps, (particularly "the drying step) needs to be qualified & controlled.
Using petri dishes with vents may also help reduce condensate issues, though vented agar plates may be more prone to desiccation depending on incubation conditions.

A6: Excellent advice from [name redacted].

I have dried plates in a laminar flow hood and used a sterile glass hockey stick with the plates on a rotary platform for spread plate methods.

Microbiological retest once a year for RTU packaging materials

RTU sterile packaging materials undergo initial receipt microbial tests.

I would like to know if there is any demand to perform microbiological retest once a year for RTU packaging materials? Do you perform annual microbiological retests for packaging materials?

Are there any guides regarding the need to perform annual microbiological retest for RTU packaging materials?

Growth Promotion Testing: MacConkey Broth

I have a query regarding the growth promotion testing of MacConkey Broth.
Growth Promotion testing is carried out in accordance with the BP (Appendix XV1 B. Microbiological Examination of Non-sterile Products).
In a recent delivery of a new batch of the media, it failed to growth promote (using E.coli ATCC 8739). Upon "retest", growth was evident however it was a much paler yellow reaction in the broth rather than a brighter yellow reaction as per usual. What could be the explanation here please? As a comparison I set up a positive control from a previously approved batch and it was evident that there was a difference in the colour of the medium. Same batch of Single Shot Bioball organism was used for all tests. Broths incubated at 42-44°C for no longer than 24 hours.

My concern is that this current delivery of MacConkey broth although clearly shows evidence of growth promotion, is not comparable to that of a previously approved batch or to my reference batch.
I have been purchasing from the same supplier. It appears that perhaps the media may have been compromised in some way.

Literature regarding Isolator (and Room) Qualification

I was wondering if any in the group could recommend sound literature regarding Isolator (and the rooms they are located in) Qualification? Some of the main items of concern are setting EM limits within them, validating VHP cycles, etc. Any information would be greatly appreciated!

A1: I recommend you read USP General Informational Chapter <1208〉 STERILITY TESTING—VALIDATION OF ISOLATOR SYSTEMS

A2: USP 1208 and PIC/s has isolator guidance. EM Limits within an isolator would be found in USP 1116 and should be <0.1%. The validator MFG should have mounds of data on how to validate the VHP cycle.

CO2 Incubator with Water Reservoir

I am having humidity issues with my CO2 incubator that has a water reservoir. During normal operations of the incubator, the humidity is set to 90%. I fill the water pan half way and the water reservoir is full. This method has been working fine for about 2 years now. I get the humidity sensor calibrated annually.

During this year's calibration I had a new water reservoir installed as the incubator was giving me alerts saying the water was low when it was not (the bobber was stuck is what they told me). Once it was replaced the incubator humidity has been too high ever since and the incubator is not responding to changes. When I called the manufacturer they told me it is because I put the water pan in there and I'm not supposed to. I am not sure how this processes worked fine for me for two years and now they are telling me I'm not supposed to have a water pan in there? Is this normal for a CO2 incubator to not have the water pan in there if it has a built in reservoir?

Use of Robotics in Sterile Manufacturing

I just read an article in the October 2021 issue of Pharmaceutical Technology about the use of robotic automation inside closed systems for the manufacturing of sterile products. The article discussed many advantages in using such a system over conventional manufacturing of aseptic products.

I was wondering whether companies are now either evaluating or starting to use robots in closed systems without human intervention for the manufacturing of sterile products.

A1: I saw a fully robotic aseptic process in Viet Nam. It was filling and processing antibiotics in IV bags. It was really remarkable.

A2: Yes, many companies are using robotic arms in glove less isolators, minus the people! There are many in some of the customers that we serve.

A3: Robotics are used in manufacturing for material transfer, e.g., loading and unloading lyophilizers. Robotics are compatible with isolators as they can be decontaminated and do not shred particles.

In lab testing robotics are used in high volume endotoxin testing and screening for drug discovery.

Low pH Products

One of our non-sterile products is a fermented extract that is sterile filtered and has a preservative added and a final pH of 3.7. From a risk perspective, would it be reasonable to reduce micro testing due to the low risk for contamination since it has such low pH, it's sterile filtered, and it contains a preservative? Any thoughts or suggestions are welcome.

A1: pH (<5.0 and >8.5) are hostile to microorganisms.

For a discussion of product physicochemical attributes that increase the hostility of a product to microorganisms would direct your attention to my book chapters.

Cundell, T. Chapter 2 - Microbial Contamination Risk Assessment in Non-sterile Drug Product Manufacturing and Risk Mitigation

and

A2: pH 3.7 is not prohibitive to microbial contamination and does not reduce relevant micro risk.

A3: It is my opinion not to reduce microbial testing on your fermented product. I suspect that your fermented product is some type of botanical. For your information, I have isolated microorganisms in products that had a pH of 3.0 to 3.5. In addition, it is common to perform a sterile filtration step in the manufacturing process of fermented products to remove the fermentation organism. I suspect that the filtrate is not being placed into sterile containers. As far of adding a preservative, it is not a justification for reducing microbial content testing. I have found that many companies are adding preservatives to fermented products in order to keep the microbial levels low because these fermented products are a good source of nutrients that will allow the growth of microorganisms.

A4: Could you please indicate how your product is finally administered ? This certainly is of influence to the answer.

HACCP / Gap Assessment against Aseptic Processing Guidelines (Initial facility assessment)

I am about to put together a HACCP and Initial Gap Assessment for a facility that produces a sterile injectable, against Aseptic Processing Guidelines (all of them). I do have some experience with this in past work, but wanted to see if any of the group has any guidance or reference materials in guiding this process. I know enough about this process to know that there's more than one legitimate way to go about this exercise, and of course aim to put together the most robust assessment possible. Any advice would be greatly appreciated.

A1: A nice guideline is PDA TR #44. Goes though FMEA examples in a sterile facility.

Purified water TOC increase during use

We use purified water as an ingredient for a non-sterile solution that is being developed.
We know that the microbiological, TOC and conductivity attributes are acceptable when the water is generated.
However, a sampling regime has been implemented where we are checking the PW attributes at specific steps during manufacture e.g. water transferred into vessel for original water quantity and then top up etc

The issue we are having is that we are seeing TOC increase above 500ppbC at these subsequent monitoring stages.
There could be a contribution to TOC levels (and we have run some trials) due to the following;

1. As it is in a cleanroom the operators are routinely spraying 70%DE to sanitise surfaces and hands and vapours in the air may absorbed during sampling the water for TOC testing.

1. The transfer tubing can have water sat in the line for a few hours whilst initial mixing is taking place before top up.

My query is that even though we are getting TOC increase above spec do we need to be concerned and be failing the water quality testing?
We have proved it is supplied of appropriate quality. if the manufacturing processes are altering the quality then we shouldn't really be trying to still monitor and pass the initial attributes as mixing ingredients will do that anyway.

Any guidance detail I can use to back up my thinking?

A1: TOC is just one of the release parameters for high quality water such as PF WFI etc. and the result along with those of other tests such as Conductivity, pH etc, reflect whether the relevant water type is of the required quality at Point of Use / Point of Generation.

TOC measurement during subsequent usage of the water as a diluent, ingredient, solvent is not necessarily appropriate or relevant - unless you are required to / find some value, for your process by controlling all contributing factors.
 In an open atmosphere CO2 will be absorbed and the TOC will naturally rise. This happens rapidly enough to be a common cause of error with release testing of the water itself, So you can imagine that any post -usage handling is bound to a include higher TOC. Ingredients may or may not contribute to TOC values and so Water Release Specification is no longer relevant.

if you are not controlling your process with respect to possible Organic Carbon sources and / or the system is not ultraclean, there is little to be gained from monitoring TOC in my opinion.

A2: For the manufacture of non-sterile products the use TOC is not required nor will it help in any microbiological studies you have.

I would suggest that you look to change any method that requires this type of analysis through your QA change control system.

Your water quality can be determined through Pharmacopoeial methods of filtration and enumeration alone.

A3: First of all let me know whether you have online TOC in return loop?

A4: You should find significant history in the files on Purified Water measurements and associated comments on the PMF website. As Derek mentioned, CO2 uptake can significantly increase TOC values. Once your Water is in use, you must adjust your measurements and specifications accordingly.

Yes, your environment can help increase the TOC value, but as Derek mentioned, that increase will be attributed more to other factors, such as CO2 uptake. Please read the history files on the PMF website. I think you will find some significant information for your internal files and records, as this issue has been asked often.

I'm also concerned that your routine water system is delivering water near the limit you listed. Please read the USP carefully and review your specifications. There are many past citations on the PMF website in the history files.

A5: Is there any statements that i can refer to that shows that i only need to look at the point of use/ generation and that the subsequent use steps i am monitoring are not required. i am trying to explain this to QA that we cant keep the quality the same as when it was first generated whilst we are using it.

The main issue is that water drawn from the system is stored in a vessel and transferred to a small manufacturing vessel as required during formulation and water addition steps. It is when we are checking the water being added 1-3 hrs later that the TOC is altering. In my opinion and following investigations, due to alcohol sprays being used to sanitise hands/surfaces etc.

We have proved that water stored in this vessel is good quality for 8 hrs, with simulated usage and we were extremely careful during sampling of this to make sure we didn't contaminate, however when being used in manufacturing and all the activities (people, sanitisations, transfer tubing) we see the change.

A6: Can you please point to me where i can identify that TOC monitoring is not required for non-sterile product manufacture. This sounds like it could be useful information for me in this situation and potentially future ones.

A7: Although it would be pleasurable indeed to scrawl through every aspect of each Pharmacopoeia to show you that it doesn't say that you have to.

I will refrain and revert your question to, why are you using TOC in anything other than previous product or disinfectant residue detection as part of your cleaning validation.

Simply put, you cannot quantify TOC recovery levels neither can you ever be sure of their actual source.

Simple enumeration requires filtration or the pour plate method, in a non-sterile environment you are unlikely to need anything more stringent.

The answer to your question is therefore unfortunately the question why are you looking for TOC as part of your non sterile product assessment criteria.

A8: Going back to the original questions (and some others that were raised), here are some different thoughts that I hope get to your issue.

1. There is some discussion about the use of Purified Water in your application. In my opinion, there is no discussion. If your registration or SOP calls for the use of Purified Water, then that is the requirement. Maybe PW could be replaced with lesser grade someday, but that is not addressing your question.

2. Ergo, if PW is required to be used, then a TOC test is a requirement also. TOC is an element of the PW monograph.

3. To address your point - The fundamental requirement for QC tests (like TOC) are to test the water 'as it is used in Production'. This can be found in USP <1231>, section 6.1.2. QC Sampling. I select the first sentence, but you should read it all. "QC sampling is intended to reflect the quality of water that is being used."

That being said, if you can make the argument that the TOC of the PW is compliant as it is used, i.e., in-line before distribution to vessel, then I would argue that your water is TOC compliant. TOC monitoring in processes further downstream are superfluous to the fundamental question "is my water compliant". I would argue that added TOC measurements are more a reflection of your manufacturing process, not the water.

However, if there is something like IPA in the environment, I would be less concerned about the TOC in the water. I would want to be prepared to answer the question "does the IPA (if it is IPA) impact the product?". Also, while your suggestions of the sources of TOC (IPA, tubing) are very possible, another is the vessel. Depending on the type of vessel, even glass, this can be significantly impactful to a TOC measurement.

A9: We are not using TOC for any cleaning verification or as part of the product assessment. It is purely to show that PW of the required quality is being used. When the water is generated, it is collected within a 100L vessel and taken to where we are manufacturing the product (small scale). We sample for micro TVC, TOC and conductivity prior to drawing any water from this vessel to show that it meets PW requirements. Which it does and we have proven that water stored within this vessel and drawn off over 8 hours also still meets all parameters.

The issue is that QA want to take samples of the PW within the manufacturing system e.g. from points of the vessel and take off points during water addition steps. It is these samples that are showing high TOC, but still meeting conductivity and micro TVC. This is where i believe that we are having environmental impact by alcohols spray/residues in the air or take off locations (after leaving to evaporate for 30 minutes) or possibly CO2 (as it is not a sealed system).

We have proved the water stored within the 100L vessel is suitable for use for up to 8 hours, but fail when checking the water quality at the in use monitoring points of the system.

These are the failed TOC results that i am trying to mitigate and remove as they are giving false indication and not representative of the water quality. We have proven initial water quality being supplied is acceptable. I cant convince QA that it doesn't matter that there is some chemical contamination or even possible changes to the water attributes due to the manufacturing environment. I need to justify the failed result sand remove the continued monitoring at these locations as not necessary. The thinking by them is that it is still PW and must meet the required parameters.

A10: I meant simply that TOC as an indication of Microbiological purity is not required not reasonable to rely upon.

TOC for chemical purity is expected.

Sorry for the confusion to you and the rest of the PMF List.

A11: The first issue is whether the PW System including the distribution system is producing PW that meets the USP requirement especially for TOC. Many companies have moved to frequent inline PW monitoring as it is routinely observed that the TOC lab measurements are higher than the inline measurement due to sampling, storage and delayed testing.I would accept a to deliver TOC standard.

The second issue is product cross contamination. When PW contacts processing equipment like tanks and tubing, chemical residues will increase the TOC levels. The equipment cleaning should be addressed in your cleaning validation program which would have TOC acceptance requirements for equipment PW rinses.

I have observed a trend for using IPA rinses as a substitute for equipment draining and air drying. In my opinion this has become excessive.

This is not my area of expertise but unlike benzene residues found in recently recalled products which is a class 1 residual solvent in USP <467>, IPA (2-propanol) is a class 3 residual solvent and may be present in drug products at a higher level. 2 ppm verse 0.5%. Benzene is a known carcinogen whereas IPA has a low toxicity.

A12: USP <1231> classifies pharmaceutical waters as Bulk Waters and Packaged Waters, where bulk water is typically produced on site i.e. is the product of the High Purity Water Generation System.

I would think finding an explicit statement to that effect in any regulatory documents is unlikely essentially because held Bulk Water and Packaged Water may or may not be required to meet the release spec. It depends on your specific operation / process / product.

No definite distinction is made between Bulk Water at time of generation or during subsequent usage, though varied handing and usage is anticipated. Obviously, the handling and usage of generated Bulk PW/WFI should take place under controlled conditions, appropriate to the intended use, that's all the Pharmacopeia asks for.

Once the Bulk is used / modified the specification might no longer be relevant.

Should the prescribed controls and related acceptance criteria of generated Bulk Water be the same as those applied to the high Purity Water Generation System?
It doesn't say so, but one could assume that if you call it Bulk PW, it should meet that requirement, but is that requirement relevant to its actual usage? -
Could you call it "Process Water" or whatever and provide another specification?

Changes in TOC / Conductivity of processed / packaged waters can often occur due to leaching from Packaging materials / processing environment. TOC / Conductivity being non-specific tests will detect such changes but not indicate what analytes are involved or their possible source. Packaged Waters may not

meet Bulk Specification and may or may not be suitable for intended use. This would be determined by additional testing.

Although long since deleted the Packaged Monographed Waters, section of USP <1231> from USP29 is informative and was at at a time when TOC / Conductivity testing was been advocated.

"The chemical attributes of these waters are still defined primarily by the wet chemistry methods and specifications similar to those formerly used for the bulk pharmaceutical waters prior to their replacement with water conductivity and total organic carbon (TOC). It is the user's responsibility to ensure fitness for use of this article when used in manufacturing, clinical, or analytical applications where the purer bulk form of the water is indicated".

As I see it you can do one of three things.

1) Engineer a suitably controlled local environment for your held water, that is equivalent to that of your Water Generation System area, as you presumably have no issue with adventitious contamination (Alcohol, CO2 etc) for those routine QC points(also PC sample points) of the Generation & Distribution System.

2) If TOC / Conductivity is not relevant to your water for its intended use, re-designate the held water e.g., "Process Water" etc and apply a new spec to it - obviously this involves suitable Change Control Approval.

3) If possible close loop the Testing Regime by conducting confirmatory tests on the TOC / Conductivity fails using the older wet chemistry tests (Oxidisable Substances, Heavy Metals , Inorganics etc. as relevant) i.e. those substituted by TOC / Conductivity . This strategy might allow you to identify that it was environmental interference rather than innate water quality changes that were involved.

Exploding Product - Are Microorganisms Implicated?

Product Recall: Why would a bottle of 240 powder-filled capsules containing Activated Charcoal from Hardwoods 2400 mg, Vitamin C 1000 mg, Aged Garlic 600 mg, Beta Carotene 20 mg.explode when opened?

A1: When you say explode, what exactly do you mean?

I presume it was a glass bottle. What was the closure configuration?

These must be very large capsules to contain those quantities of components. Were there any excipients in the fill?

If it is a dry product, I would assume that the water activity was too low for microbial growth, unless the contents of the bottle were contaminated with water in some way prior to sealing.

I would also check for a reaction between components. Ascorbic acid is labile, even in the solid state, under some circumstances. Is it interacting with e.g. a component of the aged garlic?

Were the capsules packed under nitrogen, for stability enhancement, and was the gas pressure too high? I think this is not likely, but I mention it for completeness.

A2: What chemicals were used? I’m wondering chemistry over microbiology. Did we have CO2 release from oxidation reaction? Thoughts?

A3: I once watched a news broadcast featuring a head aviation investigator talking about an aircraft incident.

This guy stood in front of the camera's and said (in his most knowing, unknowing voice).

"From time to time for no particular reason aeroplanes just fall out of the sky".

I most thorough investigation indeed, but what he was trying to portray was that statistical anomalies (what we in the business like to call outliers) do occur.

In your case, and depending on the glass manufacturer, small fractures are a known phenomenon.

From time to time and we are talking 1 in 10 million perhaps, those small fractures align in such a way that pressure (on opening) can cause a type of 'explosive' reaction.

As you say, single use low Aw containers are unlikely to have any microbial activity.

It just seems that you're a microbiologist looking for a microbiological answer.

A4: This lame aviation investigator should get another job. There is always a reason why a bottle explodes or an aircraft falls from the sky. He has just not found it!

A5: The ascorbic acid when degrades form carbon dioxide. Since bottle was sealed same thing might have happened. I have experience the same during carrying out stability of Vitamin C injection in ampule form.

A6: If you had applied a microorganism directly, that would have given a simple explanation relating to the degrading principle but considering the following: i. cross contamination by filamentous microorganisms and ii. presence of microbes in the substrate would answer your question; Are Microorganisms Implicated? What is important is the aim and objective(s) of your experiment because if properly justified, you will be able to eliminate the factor(s) responsible for the observed outcome. So, take time to revalidate your protocols and determine the status of each material used during the investigation.

A7: Thank goodness somebody didn’t eat those capsules. That much charcoal and garlic would make any human explode!

The irony that something designed to be an intestinal/colon cleanser is bursting the containers is ironic.

Hardwoods and charcoal are highly hygroscopic. I assume there was a moisture control issue and it cause the capsules to swell. Or easier answer, they were stored improperly and rapid cooling and heating occurred.

I’ll go back to my corner!

A8: I would have to think that this was a joke but years ago, I had a client who made sodium bicarbonate in sealed pouches. The pouches were swelling and they didn't know why. I simply took a gas tight syringe, pulled the gas from the package and did a gas phase infrared spectrum. The only peaks in the spectrum were correlated to carbon dioxide. Activated charcoal is used as a carbon dioxide scrubber, so may be a source of carbon dioxide. The response by Anand Borkar would be easy to prove. Just do a gas phase infrared spectrum. This is an easy test to perform.

Validating sterility of sampling containers

Can you help with this:
How can we validate the time the container maintains sterile after sterilisation by dry heat?
Do we need positive control and if we do, what would that positive control be/how would you test it?

For example: we want to prove our containers are sterile after sterilisation by dry heat (2 hours, 160°C; 30 minutes, 180°C) and that they can be used as sterile for one month after sterilisation.

In general: sterility of the containers and utilities used for sampling - validation, guidelines?

A1: When I visited the Pasteur Institute I observed Pasteur's swan-neck media containers that were apparently still sterile after approaching 2 hundred years.

You need to pick a reasonable hold time and qualify it by sterility testing.

2 weeks may be a suitable time.

A2: You need to demonstrate the sterility at different time interval and then you can fix a timeline for usage of the container as per your requirements.

oil testing

We are struggling in filtering the bioburden sample of hormone (non aqueous product) due to viscous product. So please guide me regarding dilution quantity of Isopropyl myristate to be taken for filtering the product.

A1: It just depends on the product...it could be 25mL or 125mL. We do have that in a sterile format for bioburden/sterility testing.

NaOH in order to remediate the possibility of Biofilm

What is the preferred concentration of NaOH to use in order to remediate the possibility of Biofilm? At what temperature? 60ºC?

A1: Ozonated water works really well. It is smaller than most chemicals and leaves no residue.

Practical guide for Non-sterile manufacturing

Recently, i've read "Practical guide for Non-sterile manufacturing" by David Roesti and Marcel Goverde. Part 5.7.2 Method Suitability for microbial enumeration tests, 5.7.2.1 Membrane filtration.

There's example of procedure where said "filter 10 ml of the inoculated product diluent ...1 g product contains 30-100 CFU".

I couldn't understand why, because i think 1 g contains 300-1000 according to the previous sentences.

I'll be appreciated for your help. Print screens of book's pages are attached below.

Example of a procedure for a 1 : 10 dilution.

- Weigh in a sterile glass bottle 10 g or 10 ml of the filterable product (Figure 5.3).
- Add 90 ml of the suitable diluent (e.g. buffered sodium chloride–peptone solution). Mix and/or let dissolve the product.

- In parallel, fill a control bottle with 100 ml diluent (e.g. buffered sodium chloride–peptone solution).
- Add in each of 5 containers (1 per test microorganism) 10 ml of the dissolved product.
- Spike each container with the diluted product and control bottle with 0.1 ml of a microbial inoculum that contains 3,000–10,000 CFU/ml test microorganism (→microbial suspension volume = 1%).
- Filter 10 ml of the inoculated product diluent immediately (→1 g of product containing 30–100 CFU microorganisms are filtered).

A1: I'm not sure I am much help but I do agree with you...

The product was prepared by making a 1:10 dilution so for every 10mL of diluted product, there is 1g of product. So the step stating to add 10mL of dissolved product to a container (lets call it A) = 1g of product in 10mL of solution.
You are then adding 0.1mL of 3000-10000 CFU/mL to A. Since you are only inoculating with 0.1mL, you are spiking with 300-1000 CFU. Now A contains 1g of product, 300-1000CFU in 10mL of solution (10.1mL if you want to be thorough).
It then states to filter 10mL immediately and that would equal 1g of product and 30-100CFU. I agree with you that you would have 300-1000CFU instead.

I thought maybe the container to which you "Add in each of 5 containers" was to already contain 90mL of the same buffer and that wasn't shown in the screenshots you sent. But if that were the case, you then would have 0.1g of product and 30-100CFU.

A2: If each container of 5 containers was already has 90 ml of the buffer , there will be 0,1 g (not 1 g) of the product and 30-100 CFU. It's not correct, as well;)
Secondly, I didn't understand for what's reason "rinse 1-3 times with 100 ml solution" after inoculation.

A3: The rinse is to ensure everything is washed down from the sides of the filtration funnel. This is a common practice when filtering and in many cases, it is the 3rd rinse that actually gets inoculated rather than the diluted product itself.

A4: Ok, i can rinse 1-3 times with buffer solution after inoculation or inoculate the 3rd rinse. Are both version correct?

Conductivity sample containers

We are having trouble finding the HDPE containers that we use for sampling WFI for conductivity testing. Can anyone tell me if PETG is an acceptable alternative? I am concerned that some of our WFI samples are collected at temperatures > 70°C which may cause a breakdown of the container material. Is it possible that leachables from the PETG could affect the conductivity results?

A1: I have used PETG for conductivity sampling in the past with no measurable impact to the conductivity - but at room temp, not 70°C. I suspect impact is negligible because of its organic nature and usage in medical applications. Of course, doing a side-by-side conductivity test with HDPE and PETG containers will tell you the answer. I suspect there is little difference, and the increase in conductivity (due to CO2 uptake) will be much larger than any distinction between PETG and HDPE. On line conductivity is much better as there are no interferences from environment, humans, sampling.

FYI - PETG from US Plastic rated -40 to +70°C. Maybe a risk for hot water.

Malt Extract Broth

I would like to formulate Malt Extract Broth (MEB) instead of purchasing the BD formulation to reduce the cost of the material. The BD media bottle lists the basic components (Malt Extract 6g/L, Maltose 1.8g/L, Dextrose 6g/L, Yeast Extract 1.2g/L) in their formulation. Do you have any good references for formulating this media?

A1: I would suggest keeping individual components purchased, and constitutes when you are making batches of media. Pre-mixing the components like any suppliers[HiMedia or BD] is not desirable, unless you want to take care of moisture in your jars and sterility etc. In 'olden' days, we used to [some labs in developing countries even today] make media from the 'scratch', add/autoclave and prepare poured media and store. Nothing wrong with it.

A2: I'm curious…. You have a formulation you provided. Did you need to look for an alternative?

If you wanted to know how to prepare it (autoclave cycle, mixing, etc.), you can always use Ronald M. Atlas, Handbook of Microbiological Media as an excellent guidance.

Having said that, for something as simple as MEB, the cost you will incur with labor and quality control will far outweigh the cost of purchasing it Ready to Use.

A3: The instructions are in the Difco Manual.

The formula is:

MALTOSE, TECHNICAL 12.75G, DEXTRIN 2.75G, GLYCEROL 2.35G, PEPTONE O.78G AND AGAR 15.0G.

Suspend the 33.6g of powder in 1 L of purified water, bring to the boil for 1 minute on a stirring hot plate to dissolve the powder.

Dispense into 250 ml bottles and autoclave at 121C for 15 minutes, Store in a water bath at 45-50 C and pour the plates..

Like [name redacted] recommends it may be more cost effective to purchase the media.

A4: Be careful regarding theoretical formulas provided by any manufacturer vs. the real formulation in production. All manufacturer's use a disclaimer to indicate that "formulations may be different in order to optimize results" and may vary due to differences in raw materials used. What BD or any other manufacturer indicates on a technical data sheet or catalog is rarely what is actually inside. This is due partly to industrial proprietary reasons but also to insure consistent performance.

In the end, I would agree with [name redacted] post that you'll end up spending more money in the long run and I would add that part of the added value in purchasing from a major manufacturer is to insure consistency. A manufacturer like BD or Solabia (my company), will mix and blend components to the same

particle size in order to insure proper homogeneity of the raw materials together : when you do it yourself, you will never achieve the same particle size and this can impact, over time, the consistency of your results. You may achieve a decent media by making it up like a cookbook (assuming, of course you all the necessary ingredients), but will you achieve consistent results over time ? Unlikely.

A5: I understand because I worked in the media manufacturing industry. With naturally source material like peptone minor adjustment may be made with the bottom line the growth promotion requirement.

USP <60> and <62> contain the following statement: RECOMMENDED SOLUTIONS AND CULTURE MEDIA [Note—This section is given for information.] The following solutions and culture media have been found satisfactory for the purposes for which they are prescribed in the test for microbial contamination in the Pharmacopeia. Other media may be used provided that their suitability can be demonstrated.

A6: Absolutely agree. Peptones are one biologically variable raw material that might require some adjustments in formulation (Solabia is a peptone producer), although we've worked on that batch to batch consistency issue which has dogged peptones historically. At any rate, USP, EU and all Pharmacopeias say roughly the same thing about equivalent formulations being used, provided you do the leg work to demonstrate the equivalence in your application. This isn't usually a big deal, but you do have to have at least some data. And the theoretical formulas are the basis of the media, and will revolve around them. But that might not be all that's there.

A7: I think that people tend to forget such classic literature as the DIFCO manual.

A8: Let's not lose sight of the fact that the DIFCO Manual is a commercial/marketing document just like any other catalogue. That it had (past tense, let's not equate historical Difco with the merger with BD of today) been considered a sort of "bible" for culture media is one thing, and one we can pretty much all agree, but the Difco Manual was not an official microbiology text emanating from an official agency but a private company's marketing tool. As such, it used the same verbiage, techniques and disclaimers that any other technical catalogue would use today. It even states, in its forward, that it has never been intended to replace any official compendium....

Difco would no more reveal trade secrets or exact media formulations in its Manual than any other manufacturer, so the same principles that apply to the Manual as any other, even if they don't specifically make the disclaimer. So whether the exact composition of MEB from Difco is the mirror of what is stated in the DIFCO Manual on page 299 is really anyone's guess but if you believe that, I may have a bridge in Brooklyn for sale as well.

Otic products

i would like to ask about the interpretation of the following- USP<4> MUCOSAL DRUG PRODUCTS-PRODUCT QUALITY TESTS-excerpt.
OTIC ROUTE
"Typically, sterility is required where the product is administered to the inner ear or where the eardrum is damaged. Where sterility is not required, the quantitative enumeration of mesophilic bacteria and fungi that grow under anaerobic conditions, Microbial Enumeration Tests (61), or the determination
of the absence or limited occurrence of specified organisms, Tests for Specified Microorganisms (62), may be required."

The word anaerobic conditions what doesn't it mean in the context here . The usual analysis as per USP<61> is aerobic condition- TAMC(Total aerobic microbial count).

when I checked USP<1111> , it sates that for auricular products the limit for TAMC is 10^2 and TYMC is 10 Cfu/ml or gm.

So , as I see there is a contradiction here or do i missing something.

Validation of Expired Media

Regarding the expired media in the Microbiology lab. If we want to use the expired media then what is the procedure, Is there any reference for using the expired media?

A1: Why would you use expired media?

A2: I do not want to throw. I want to use it. It's still working. Passes gp, indicative, inhibitory tests. That is why I am asking. Also want to know, how the media will work for certain period & why it will not work after that period.

A3: On the rare instance when it may have been necessary to use expired media, an additional growth promotion test performed on the expired media, on the same day as it will be used for the test in question has been considered defensible.

A4: I would not recommend using expired media. My only comment would be that I have performed post-expiry growth promotion testing, where we hold lots of media 2 weeks after the expiration date and perform full growth promotion testing. This data was not collected with the intent to justify using expired media however, more so to justify using media up to the date of expiry, where the it may technically expire during incubation.

A5: I would submit that in order to demonstrate growth promotion for expired media (which, by the way, should never have to be performed if the lab plans accordingly), one should perform growth promotion on the LAST day of incubation. This way, growth promotion has been demonstrated throughout the actual use period of the media and not just when the incubation period starts.

A6: I have tested media that was a few years old and it still passed GP (room temp stable media).

Just because you could use expired reagents/media doesn't mean you should. Use of beyond use date should be reserved for emergencies, when no other options exist and an appropriate deviation should be written with a CAPA to ensure it stops happening.

You seem to want to make this a rule, rather than an exception.

Why does media stop working? Generally because the media has to not only support the assay (air sampling, MLT, bioburden, etc) itself but the entire incubation period as well. Older media already has moisture loss and that will cause the media to be too desiccated to support growth at the end of incubation.

Simple answer? There's magic dust in the media. That powder only lasts until the day of expiration. It's sorcery more than science.

I'm sure I have a container of milk that expired last month. Smells fine, but I can't bring myself to take a swig.

A7: Be a rebel. Some media might survive past the expiration date, others won't, hence the blanket expiration. There are some media that are very hygroscopic and if opened multiple times will not last it's entire shelf-life. Other media will have components that breakdown as well.

A8: Yes, I Agree , It would only be acceptable for exceptional circumstances with full deviation / investigation record / CAPA etc.

Routine practice of using expired media would be frowned upon by most if not all GMP inspectors and offer them ample opportunity to drill down, something to be avoided.

The critical supporting data IMO would be that it passes both GPT conducted in parallel with the test sample (samples) and a post-test GPT, following completion of the test sample run. Effectively, this would equate to x 3 the amount of routine GPT workload, - reason enough for not using expired media on a routine basis, just in case keeping the right side of GMP isn't a good enough one.

A9: One should not use expired media at any cost. Why you want to use expired media, now a day ready to use media plates, bottles are easily available. You can perform parallel GPT and Test as well.

A10: I have found that under emergency conditions, maybe two or three times in 40 years, using expired media if you preform a growth promotion side by side, has been acceptable. Yes, bottles are easily available, but there may be extenuating circumstances under which a test must be run, and all you have is expired media. By running side by side growth promotion testing that passes, you are proving that the media will grow microorganisms.

RWC- PHENOL CO-EFFICIENT

Can anyone help me with step by step procedure for RIDEAL WALKER CO-EFFICIENT test / phenol co-efficient test ?

A1: Phenol coefficient method is a measure of the bactericidal activity for a chemical compound in relation to phenol. I will admit that it has been too many years in which I had to perform either the phenol coefficient or the Rideal-Walker coefficient methods for a disinfectant and I'm not a big fan of either. It should be noted that the Rideal-Walker phenol coefficient method had been developed in 1903 and is very similar to the phenol coefficient method, but it is different.

To calculate phenol coefficient, the concentration of phenol at which the compound kills a test organism in 10 minutes, is divided by the concentration of the test compound that kills the same test organism under the same conditions (or, probably more common, dividing the dilution factor at which the tested substance shows activity by the dilution factor at which phenol shows comparable activity). A phenol coefficient of 1.0 means that the chemical agent has about the same level of effectiveness as phenol. A chemical agent with a phenol coefficient of less than 1.0 is less effective than phenol.

In the Rideal-Walker method, the phenol coefficient is the ratio of the dilution of the disinfectant that kills the test organism to the phenol dilution that kills the organism at the same time under the same identical conditions. Phenol solutions of known strengths are used; cultures are grown in a standard microbial growth medium, sub-cultures are made every 24 hours; sterile loops used for all inoculations are of a standard size (about 4 millimeters in diameter). Dilutions of suitable strengths of the disinfectant to be used are prepared by using sterile deionized water (i.e: 1:100. 1:200, 1:250, 1:300, 1:350, and 1:400). Phenol test controls of a suitable strength are also prepared by dissolving 1 gram phenol in 95, 100, 105, 155 milliliters of sterile deionized water. Five-milliliters of each of these dilutions are placed in sterile test tubes, to which are added at 30-second intervals by using a 24-hour broth culture of Salmonella typhi (NCTC 786) in the proportion of 1 drop of culture to each milliliter of disinfectant used. At 2.5-minute intervals, a loopful of each of the mixtures are inoculated into separate test tubes containing 5.0-milliliters of standard microbial growth broth, an interval of half a minute being thus allowed between taking the samples from each of the different dilutions.

This is repeated at 5, 7.5, 10, 12.5 and 15 minutes. The inoculated broth tubes, after being incubated at 37" C. for 48 hours, are examined for the presence or absence of microbial growth. Let's say at 7.5 minutes of exposure, the test organism is killed by the phenol solution at a dilution of 1:100. At the same time-period of 7.5 minutes exposure for phenol, the test organism was killed by using a 1:250 dilution of the disinfectant. The phenol coefficient is calculated by dividing 250 by 100 which is 2.5. This result indicates that the test disinfectant can be diluted 2.5 times as much as phenol and still possess equivalent killing power against that test organism. Disadvantages of the Rideal-Walker test areas follows: No organic matter is included; the Salmonella typhi test microorganism may not be appropriate; and the time allowed for disinfection is short).

However, it should be noted that the Chick Martin test is a modification of Rideal–Walker test method, in which the disinfectant acts in the presence of organic contaminants (e.g., dried yeast, feces, etc.) to simulate the natural conditions.

RODAC (SDA LP80) shortage

We are seeing an industry shortage for RODAC plates (as with many other items involving polystyrene) - both our main suppliers, BD and ThermoSci are continually backordered for SDA LP80 RODACS. Does anyone have alternate supplier suggestions?

Unusual Colony Help

I am hoping to get some help with information on an unusual colony that we have recovered from our facility. We had a similar looking colony a few weeks ago that we sent to Accugenix for ID and they were unable to obtain a result. We have now received a second monitoring plate with colonies of this appearance. We wanted to gather as much information as possible before attempting to ID with Accugenix. Therefore, I planned to perform a Gram stain and sub-culture. When I tried to pluck part of the colony from the plate, I found it to be hard and unable to be divided. Eventually the entire colony lifted, whole, off the surface of the agar. Has anyone ever seen anything like this? If anyone has an insight, it would be greatly appreciated if you would share any knowledge that you have. If it is relevant, we are filling Vancomycin HCl vials for lyophilization. This growth was recovered from those needles post fill. Thanks!

A1: Yes, it sounds like it could be an actinomycete.

A2: 1. Prepare a 2% NaCl suspension, dispense the hard colony into it and shake vigorously. Incubate at differential temperatures (30 – 45 oC). Take 0.5 ml of the suspension and use for fresh isolation using Nutrient Agar or Potato Dextrose Agar or Blood Agar.

OR

2. Add 2 ml of water or glycerol onto the plate OR add 3 ml of water or glycerol into the vial. keep in the incubator for 15 minutes at 30 oC. At the end of incubation, take 0.5 ml of the suspension and carry out fresh isolation.

Hardness of colony is usually caused by stress factors resulting from limitation of nutrients especially in agar storage media for a long period.

Try the above techniques to recover the strain and then apply wet mount technique (for mold microscopy) or Gram staining (for bacterial microscopy) to further confirm purity. Afterward send for molecular identification.

A3: Yes, sounds like an Actinomycete. Very interesting group of organisms commonly found in soil. We've studied them for anti-microbial properties as well as their ability to use hydrocarbons as a food source. Strange looking guys!

A4: The fact that Accugenics could not identify, indicates the 'colony' or the 'growth' which is dry and crusty appears to be some form of crystal formation in the media reacting to your product. Did you check its microscopic appearance? What was it like?

What was the media in which you observed it? If you change media do you still get such 'growth?

USP 61 and 62

Is it required 10g of sample for testing only TAMC and TYMC for Non-sterile API. Or can we use 1g of sample which was performed and observed recovery during suitability test.

In USP general chapter <61> and 62 (Microbiological Examination of Non-sterile products: Microbial Enumeration test and specified microorganisms) mentioned Suitability of counting method in presence of product mentioned that dissolve or dilute (usually 1 in 10 dilution is prepared).in this section sample quantity is not specified, if there is no recovery we have to go for higher dilution for method suitability.

In TESTING OF THE PRODUCT, amount used for the test mentioned as 10 g of sample. For materials used as active substances where the sample Quantity is limited or batch size is Extremely small(I,e less than 1000 ml or 1000g) the amount tested shall be 1% of the batch unless a lesser amount is prescribed or justified and authorized. Examination of the product: Prepare the sample using a method that has been shown to be suitable as described in growth promotion test and Suitability of counting method. there is ambiguity regarding sample quantity.

During specified microorganism testing procedure, sample preparation reference mentioned <61> this mean that sample quantity mentioned in 61 chapter is for 61 and 62 chapter tests purpose.

Some of the API having near 1 kg batch size and having very high cost where sample quantity unable to provide 10g can we use 1g of sample for TAMC and TYMC (same dilution i.e 1: 10).

We consider 10g of sample is mandatary for TAMC and TYMC during method suitability some of the API (insoluble) having inhibition at 1:10 ,1:50 and getting recovery at 1:100 dilution.

Sample preparation for 1:100 dilution:

1. 10g of the product + 990 mL diluent is not feasible for handling i.e. mixing, pipetting, area occupation and sterilization.
2. 1g of the product + 99 mL diluent is feasible for handling i.e. mixing, pipetting, area occupation and sterilization

Based on the above information is it to consider 1g / 10g of the product for testing microbial enumeration test?

If any one having different approaches to minimize the sample quantity please provide.

A1: USP <61> provides a level of flexibility for API testing, allowing a single sample of not less than 1% of the API batch rather than the standard requirement of 10g/10 ml.

Further test reduction is possible, but only if you can justify that a smaller amount does not constitute a reduction in the effectiveness of product testing used to assess quality and assure patient safety. Any change to test regimen would have to be submitted as a supplement to the relevant regulatory authority and most probably be subject to prior approval.

Without prior precedence it's unlikely that antimicrobial activity alone would provide sufficient justification for reduced release testing. Microbial risk is not the only potential issue - are there other CQAs that might not be suitably established or assured using a smaller sample size ?

Any submission should include a suitably comprehensive Risk Assessment that includes but is not limited to factors such as API synthesis, source, supplier history, test suitability (LOD,LOQ), QC test history (impurity profiles, & stability) available literature in the public domain, final dosage format (liquid / solid, sterile non-sterile etc,) final concentration etc etc. The level of additional input would very much depend on how comprehensive the original CMC / CTD submission documentation was.

Your QA /RA departments should be able to support you in determining the nature & content of the required supplement.

The Power of the Dog (Spoiler Alert)

In Jane Campion's movie in a surprise ending anthrax spores are used to commit the perfect murder. From a microbiological point-of-view is the crime feasible?

A1: Thanks [name redacted] - don't know but you'll recall shaving brushes as fomites. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5403022/
Suppose this speaks to old guys like myself but wonder how many have read Arrowsmith by Sinclair Lewis (of Main Street, Babbitt, etc.) - a microbiologist investigating a plague, won a Pulitzer. Paul De Cruif (Univ Chicago - author of Microbe Hunters) claimed to have coauthored Arrowsmith - sued Lewis and lost.Think microbiologists would enjoy Arrowsmith but certainly should have read Microbe Hunters.

A2: Haven't seen it. I still haven't recovered from the scientific inaccuracies of Cuba Gooding Jr and Dustin Hoffman's roles in Outbreak.

A3: Does anyone remember the PBS series Mystery .... they did a show Malice of Forethought (based upon the book by Anthony Berkeley Cox) ....as I recall a husband tried to kill his wife using Salmonella ...

pH and growth promotion for Antibiotic media

I would like to know how colleagues are adjusting, if necessary, the post sterilization pH for antibiotic media used for bioassays. A company I am associated with, is essentially using the pH range listed in the Difco/BD manual, which routinely has a 0.1 total range, e.g. for Antibiotic 11 media is 7.95 +/-. 0.5. The range is also different from what is listed in USP <81>, which is higher than the Difco/BD documents, and has a 0.4 total range . In general, what are companies doing if the value is not in that range? Is it automatically discarded. A main issue is that being an agar media, in order to take the pH at 25 degrees C, it is solidified and can't be adjusted at that point. Are companies doing studies to verify as best as possible that a pre sterilization pH value changes after sterilization into the needed post sterilization 25 degrees C pH reading. Also, what are people doing for growth promotion organism(s) for e.g. Antibiotic media 11. The organism used for the assay test is M. luteus, but we are only using S.epidermidis as a growth promotion organism. Any input would be appreciated.

Antimicrobial effectiveness testing of ointments

Please be so kind to share your experience regarding antimicrobial effectiveness testing of the ointments (preservative efficacy testing).

We have noticed that aqueous inocula (as per EP 5.1.3.) "separate" themselves from the ointment samples, and result in unacceptable log reduction results - results that do not comply with the acceptance criteria, results that are not repeatable if the test is done multiple times, and results that do not make sense (for example, no growth in 2 days, 1000 cfu/g in 7 days, no growth in 14 days, 10000 cfu/g in 28 days, etc.).

I came across a CTFA M-6 Preservation testing of atypical personal care products. I do not have it, so I do not know what does it say, for which products is it applicable, which methodology is used...does anyone here uses this methodology? Can it be applied to pharmaceutical ointments?

Can you recommend any other alternative procedures in case of the ointments (alternative to the aqueous inocula described in the EP 5.1.3.)?

A1: Not a big fan for testing ointments for preservative effectiveness due to the fact that the water activity was low (e.g., 0.26 for Preparation H ointment) and the preservatives used tend to partition into the hydrocarbon phase. That said, when I was doing these type of samples, glass Petri dishes and sterile-filtered Isopropyl myristate (stored in an amber bottle) were used to process the samples.

Fungicide

USP 1072 suggests a 3 log reduction for vegetative bacterial disinfectant efficiency. A 2 log reduction for bacterial spore efficiency. Is there any recommendation i can use for fungicide log reduction?

A1: Although not described explicitly in <1072>, all the disinfectant efficacy qualification tests I have seen use 3 log reduction as "pass" criteria for all organisms other than bacterial endospores. This includes fungi (and even virus). Also, that 3-log criteria would apply to both vegetative and spore forms of fungi.

A2: <1072> is going through a revision that will include a log reduction requirement for fungal spores. Check it out online in the Pharmacopeial Forum.

A3: That is indeed quite interesting proposal in revised <1072>:

The disinfectant efficacy test must have realistic acceptance criteria. In practice, sufficient organisms need to be inoculated on a 5.08 cm × 5.08 cm square of the surface being decontaminated (i.e., a coupon) to demonstrate:
At least a 2-log reduction (for bacterial endospores and fungal spores) and
At least a 3-log reduction (for vegetative bacteria and fungi)

It will be interesting to understand how one is going to test vegetative forms of fungi separate from fungal spores. Fungal spores are so different in so many ways that bacterial spores, Im confused as how this was justified.
It also disagree that <1072> should include fungal spores in their definition of a sporicide as this does not match how fungicides are registered with EPA. While certain fungal spores are less susceptible than vegetative forms, I thought their level of inherent "resistance" pales in comparison to bacterial endospores. Curious to other's thoughts.

A4: I was puzzled and curious from your first email on concept of 'log reduction ' of Vegetative stage of fungi? Fungi typically are filamentous or yeasts that multiply by budding. How do you accomplish log growth/reduction criterion for mycelial stage which is the vegetative state. Am I missing something here?

A5: The text you refer to was copied from the draft USP <1072> document.

I found the draft on the USP-NF website, although took a while to locate.

[cid:image001.png@01D7F351.70D02A30]

I suppose they are trying to differentiate among non-spore forms of fungi (eg, hyphae/mycelium) and spores.

I too am puzzled! While it possible to isolate spores from sporulating fungi with minimal contamination from hyphae, I don’t know how one goes about testing bits of mycelium with any kind of reproducibility…

I would suggest to my customers to test against fungi like we always have and target a minimum of 3-log reduction.

Microbial limit test sample preparation

For microbial limit test, can we crush sample or sonicate to make homogenised sample solution? I hope that this is not advisable. However for delay release tablet if it is taking 2 hours to dissolve then how we can proceed further?

A1: You can use blender or stomacher to breakdown slow dissolve products if needed. Be careful about sonication. Just make sure whatever method you use, it is followed for methods suitability to ensure it is not impacting recovery of microorganisms and maintaining aseptic conditions.

A2: I know it's meant for ointments and creams but have you tried Isopropyl myristate? It's a bug-friendly solvent. We do have it as a sterile preparation or you can filter sterilize it in your lab. What do you think are the ingredients that are causing it take so long to dissolve?

A3: You can crush in sterile bag and must perform suitability test and validation.

Personnel and Grade D Monitoring

I have questions regarding Environmental monitoring, when the aseptic liquid filling is a closed Isolator System.

One of our client who will be manufacturing aseptic DP (liquid in vial) for us, doesn't have Grade B in the controlled area where the filling Isolator is. They explain to us that their Isolator is closed system and therefore there is no Grade B (Isolator is in Grade C area) hence no personnel monitoring required. Is this as per aseptic processing cGMP?

Also, their Grade D area is monitored only "At Rest" condition not monitored at "In Operation" condition. Is this as per aseptic processing cGMP?

A1: The short answer to both of your questions is yes, this is likely to meet cGMP.

The design, installation and usage of the specific isolator will determine the classification needs/ requirements of the surrounding area. In my experience it has been very unusual to build Grade B around an isolator unless a specific application required it. Personnel monitoring in the surrounding area would also be unusual unless there is some particular risk to the product or process. Most isolators have gloves and they are most appropriately considered to be part of the interior surface monitoring program, not personnel monitoring.

There is generally broad latitude for monitoring a Grade D environment. You should review the documentation for establishing the EM program to ensure it includes appropriate justification for the monitoring sites, frequency and conditions.

A2: USP 1116 states below a chart on page 7: "All operators are aseptically gowned in these environments (with the exception of background environments for isolators). These recommendations do not apply to production areas for nonsterile products or other classified environments in which fully aseptic gowns are not donned"

The FDA Guidance for Industry doesn't specifically state that the surrounding environment of an isolator needs to be measured for EM, however, many firms do test the surrounding environment periodically.

PDA Technical Report 13 doesn't recommend monitoring of personnel in this situation, but does recommend testing the gloves post-use to detect any contamination derived from pinhole leaks and integrity loss.

In the new EU Annex 1 GMP it does state that isolators should be located in a minimum Grade D environment.