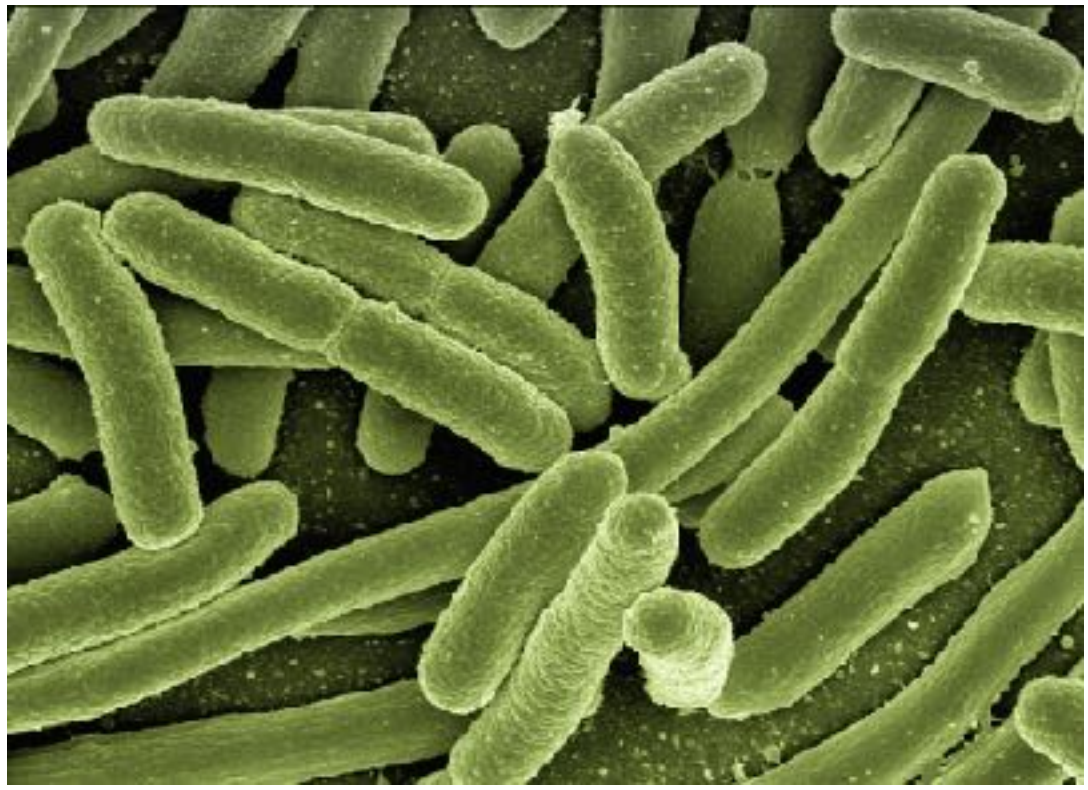
PMFList Yearbook

2020



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 2020. 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 2021 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.

Initial EMPQ

We are running our initial EMPQ of our fill room and Isolator (Isolator in a Grade C background). We have been given conflicting opinions of how many days of dynamic we need… 14 versus 3 days.
This is for a parental drug company.
Thoughts?

A1: In general we strive for 7 days to support a good trend.

endotoxin of acids

An auditor is worried about inorganic acids used in our aseptic process. The acids are sterile filtered. The acids remain in the final product in a high concentration. The questions were the risk of microbiological contamination to the acids over time of use (3 months for each lot). The request was to ; test bioburden test endotoxin. . Is it reasonable to test an acid for bioburden /endotoxin? How do you test an acid for bioburden, dilution/neutralisation ? Is it feasible? Most microbes will not survive in acids, is there any reason to test at all?

Risk assessment of non-sterile product for additional microorganisms

USP and EP chapters associated with the microbiological quality of nonsterile products list specified organisms for the different product types, however it is still expected that you conduct a risk assessment to determine if you require to test for any other organisms that could be considered objectionable to the product or patient. Also, for non-steriles as there is a new Bcc chapter you would want to state your rationale for testing or not of Bcc.

Does anybody have any guidance, or an example they could share of such a risk assessment for a product or what headings/things you think should be included within a product risk assessment?

A1: I would refer you to Technical Report No. 67 *Exclusion of Objectionable Microorganisms from Non-sterile Pharmaceutical and OTC Drug Products, Medical Devices and Cosmetics* 2014 and Cundell, T. Chapter 2 - Microbial Contamination Risk Assessment in Non-sterile Drug Product Manufacturing and Risk Mitigation and Chapter 11 - Exclusion of Objectionable Microorganisms from Non-sterile Pharmaceutical Drug Products*Pharmaceutical Microbiological Quality Assurance and Control – A Practical Guide for Non-Sterile Manufacturing*. D. Roesti and M. Goverde (editors) J. Wiley & Sons 2020 for guidance.

A2: The risk assessments I've written typically have the following sections:

- Microbiological test history for the product, raw materials (USP <51>, <61>, <62>, <60> if applicable)
- Manufacturing controls
- Prevention of contamination
- Raw materials origin and water content
- Manufacturing processes
- Intended use and route of administration of the product

I suggest you start with USP <1115> for guidance. Just briefly:

- Is the product route of administration a higher risk? (for example inhalant or nasal spray versus an oral tablet)
- Does the product target high risk populations? (neonates, immunocompromised, etc.)
- Does the final product have high water content or high water activity?
- Do the raw materials have high water content or high water activity?
- If water is used as an ingredient, what controls and monitoring are in place for the water system?

- Are the raw materials synthetic or naturally derived? (naturally derived ingredients are higher risk)
- Is the product self-preserving or are preservatives added? (for example, many topical gels have high alcohol content)
- Does the manufacturing process have steps that would lower contamination risk? (for example, mixing at high temperatures or curing the product in ovens)
- What safeguards and monitoring are in place to prevent contamination during manufacture? (gowning, hygiene program, cleaning validation for equipment, ISO classification, etc.)
- Is the product adequately protected from contamination by its container during shelf-life?

A3: Here's a good article by Scott Sutton that may help.

Search Google for: "Objectionable Organisms" - The Shifting Perspective

USP 60 Question

Does USP 60 apply to lotions and foams for cutaneous use? It seems that it would and wanted to get a little more perspective on this.

A1: If the lotion or foam is a nonsterile aqueous product formulation, USP Chapter 60 would apply.

Is Bacillus subtilis an Objectionable Microorganism?

In USP Chapter 1111, the recommended microbial limits for non-sterile aqueous drug products is a bacterial count of no more than 100 CFU/ml, a fungal count of no more than 10 CFU/ml and the absence of E. coli in 1 ml.

In December 2019, there was a product recall of a non-sterile aqueous oral drug product formulation in the United States due to use of a raw ingredient that had been contaminated with Bacillus subtilis. There was no indication in the recall notice that the aqueous oral finished product itself had been contaminated with Bacillus subtilis.

If the used raw ingredient was out of compliance with its microbial test specifications, I can fully understand as to why there was a product recall because an adulterated raw ingredient had been used in the manufacturing of the finished product formulation. In looking at the composition of the recalled product formulation, it is highly likely the raw ingredients that could be susceptible to contamination by Bacillus subtilis is either glycerin or maltitol solution. It would not be unusual for either one of these 2 raw ingredients to be contaminated with Bacillus spores. In addition, the preservative system of this product formulation consisted of methyl and propylparaben that are more antimicrobial active against fungi than bacteria.

There are several issues with this product recall. What were the microbial test specifications of the raw ingredients that are susceptible to microbial contamination? Most raw ingredient manufacturers will often have a bacterial count specification of 1000 CFU/gram or ml for their raw ingredients that are susceptible to microbial contamination. It would be unusual to see a microbial count specification of 100 CFU/gram or ml for a raw ingredient that is susceptible to microbial contamination such as glycerin or maltitol solution. Let’s say that the used contaminated raw ingredient had been used in the manufacturing of the recalled product formulation had been in compliance with its microbial test specification but was contaminated with a low acceptable level (e.g. 10 to <100 CFU/gram) of Bacillus subtilis spores. Would people consider the presence of low levels of Bacillus subtilis (e.g. 10 to <100 CFU/gram) as an objectionable organism in a raw ingredient that is used in the manufacturing of a non-sterile aqueous oral product formulation? If low levels of Bacillus subtilis is present in a non-sterile aqueous oral drug product formulation is objectionable, how do you accept foods that are orally consumed as not adulterated due to the presence of Bacillus subtilis? Besides, many foods have higher microbial count levels than non-sterile aqueous oral drug product formulations.

It seems that it is becoming more difficult to be a microbiologist in today's environment when considering whether low levels of particular microbial species in a raw ingredient is objectionable or not objectionable for a non-sterile product formulation. If you follow the information that is present in PDA TR 67, I would think that low levels of Bacillus subtilis in either a raw ingredient or a non-sterile finished product formulation would not be considered as objectionable.

A1: This recall got my attention as unnecessary and I will have an article entitled "Is Bacillus subtitis Objectionable in an Oral Solution?" appearing in the March-April issue of American Pharmaceutical Review" that discusses, in detail, the lack of justification for recalling the product when spores from B. subtilis was found in one of the ingredients. The article included a full risk assessment.

AET Burkholderia cepacia complex + wild isolates maintenance issues

inspired by the USP 60 discussions going on lately, I would like to present to you a case of a *liquid sample*, which was tested for its *antimicrobial effectiveness *(European pharmacopoeia chapter 5.1.3. test was performed, to be exact) towards *Burkholderia cepacia complex species (the so-called AET test).*

Several issues were addressed - please see them below, and please advise or share your experiences. I apologize for the amount of text.

The sample was inoculated with 100 000 - 1 000 000 cfu/ml of B. cepacia ATCC 25416 and B. lata (wild isolate). Each species was tested separately, and the test was performed on several occasions. In each time interval, cfu/ml were determined using a pour-plate method, whose suitability has been demonstrated using the ATCC strain, prior to the AET (buffered sodium chloride peptone solution for the sample preparation, tryptic soy agar for the plates).

The 0 h test (immediately after the inoculation) revealed a slight, but significant cfu/ml decrease in most of the samples (initial log reductions were in the range of 0.5-3.5, probably depending on the speed of the operator, i.e. on the time that passed between the inoculation and the initial test: the range of 5-10 minutes).

Therefore, 2 and 7 day results were no surprise: no growth was detected (<10 cfu/ml), i.e. maximal log reduction was achieved.

But, 14 and 28 day results were a BIG SURPRISE for most of the samples: the initially inoculated numbers reappeared, i.e. growth of 100 000 - 1 000 000 cfu/ml was detected.

My interpretation is that the inoculated burkholderias were damaged by the initial contact with the sample, so they were unculturable during the first week or so, but then they adapted, regained their culturability, and they were again able to grow on tryptic soy agar plates after 14 and more days after the inoculation.

Would these "no growth" results still be "no growth" with the USP 60 BCSA medium, or would this medium reveal the damaged burkholderias that did not grow on tryptic soy agar plates? Do you have any experience regarding this "silent viability"?

Also, I have to point out, that in some of the samples, culturability did not return. In other words, 28 days after the inoculation (even 65 days after the inoculation!), the result was still <10 cfu/ml. Bearing in mind the aforementioned results - *how can we be sure that these "no growth" results really meant "no growth", and not "damaged, not yet culturable"? *In these 28 and 65 days intervals we also tried the tryptic soy broth method (subcultured to a type of a "urinary tract infection" selective plate that supports growth of B. cepacia complex species), but "no growth" was also observed.

These samples in which culturability did not return were collected during AET tests performed on the same samples (same sample batch), but on different days (inoculation was done on a different day), with different "batches" of inocula, than in case of the samples discussed above in which culturability was regained after 14 days.

In my experience, most of the pharmaceutical microbiology labs carries out the AET only on one sample per test microorganism. *Our case shows that AET can show great variability, and that it should be carried out in several independent trials. Please share your opinion.*

The main concern that is raised by the results of our AET test is the routine analysis of the sample in question. Lets say the sample gets contaminated with B. cepacia complex species during production. Microbiological analysis is carried out immediately after the production...well, we can assume that the same thing happens as in the AET: the initial contact with the sample damages the cells, and they are not revealed during the routine microbiological purity test. In light of the AET results - *would it make sense to carry out the microbiological analysis not sooner than 28 days after the production?*

Of course, all of these problems would probably be solved if we had more sophisticated detection methods*. Do you have any experience regarding B. cepacia complex specific PCR, or any other more specific method that does not rely or only partially relies on the culturing steps?*

In the end, I must cite the pharmacopoeias that say that the preparation of the test microorganisms for the AET must be such that ensures *the optimal state of the organism. What does this mean? How can we know what is the optimal state?*

For example, we have noticed that if the B. cepacia inocula are prepared from the agar slant (agar slant from the refrigerator subcultured to the fresh agar slant which is rinsed with saline after incubation), they are more resistant to the sample (and therefore more appropriate for the AET) than the inocula prepared in the broth (agar slant from the refrigerator subcultured to the broth). Using only culture-based methodology, there is no way of knowing (prior to the AET) that the agar-slant preparation is more optimal than the broth preparation.

Also, in case of our B. cepacia wild isolate (B. lata) we have noticed that if it is kept on an agar slant in the refrigerator for more than 2-3 weeks - it dies off, i.e. if it is subcultured from this refrigerated agar slant to a fresh agar slant or to a broth, and incubated: nothing grows. That is why we subculture to fresh agar slant every two weeks. *Does anyone have experience regarding maintenance of the wild isolates in this way?*

One of the concerns of such culture maintenance is of course the rule of keeping the number of passages under 5. Our ATCC strains can be maintained in under 5 passages - the ATCC carrier is, for example, declared to be the 3th passage; it is rehydrated in the broth (4th passage) and then subcultured on the agar slant (5th passage) which is kept in the refrigerator and used throughout one month for growth promotion of the media, for the AET tests, for method suitability tests etc.

Once we grow a wild isolate from a sample on an agar slant and put it in the refrigerator - that is passage no. 1. *Each transfer to a fresh agar slant (one per month) is a passage, is it not? *In such a way, after 5 months, the wild isolate is no longer in under 5 passages, and can not be considered to be the same as the one that was originally isolated from the sample.* What would be a better way of maintaining wild isolates and making them more suitable for the AET tests? Would lyophilisation be a better way? *Please share your experiences.

Pharmacopoeias mention that the list of standard test microorganisms for the AET tests can be supplemented with wild isolates...how often is this really done in real life? *Do you use wild isolates for the AET tests, and how do you decide which ones should be tested? Do you maintain a record or a base of some sort, in which you can track the types of isolates, frequencies and sites of isolation...?*

A1: How DARE those little bugs refuse to follow our rules! Just who do they think they are?
Welcome to the world of microbiology. I'm not sure why Bcc reappears after being 'deactivated" - Adaptation? Genetic modification? Plasmids? Whatever the reason, it sounds like a great research project.
I'm also not sure what the implications are of your findings. Extending the time of the AET? This reminds me of the sterility test, which used to be 7 days and was ultimately extended to 14 days after reports of regrowth after 7 days. One thing is for sure - this topic will generate some great responses.

A2: The USP <51> recommendation concerning adding more species as challenge organisms is as follows:

"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."

The chapter makes no recommendation on using wild types. In fact, <51> being a standardized official test method relies the use of type strains as stated in the chapter in Table 2 with the acceptance criteria in Table 3..

When I directed a microbiology lab in early development we routinely used B. cepacia as a challenge during antimicrobial preservative system development for aqueous, multiple-use dosage forms especially nasal sprays and topical lotions and creams. With some formulations that were highly effective against S. aureus, E. coli and even P. aeruginosa were ineffective against B. cepacia. We reformulated so the product passed with B. cepacia.

The recovery of challenge organisms after a > 5 log reduction earlier in the test period at the 14 and 28 days is but uncommon and is termed the Phoenix Effect and is the rationale for taking the test out to 28 days.

A3: As Frank had indicated, welcome to the world of Microbiology. In reviewing your post, I believe that the problem with your initial challenge test results is improper neutralization of the preservative system of the test sample. For example, I have included the usage of Tween 20 and Soy Lecithin as preservative neutralizers in the diluent of Buffeted NaCl Peptone Solution with good results for the recovery of Burkholderia cepacia ATCC 25416 and wild-type isolates. Without proper preservative neutralization, you may inhibited the growth of the challenge test organism by the preservative system causing a sub-lethal injury that does not allow for initial recovery.

As far as the Phoenix effect, it does not exist from my many years of experience in conducting challenge testing. It is a poor explanation of why there was a challenge test failure. In having the-growth of a challenge organism in a test sample, there was either improper neutralization of the preservative and the preservative might not be cidal against Burkholderia cepacia complex isolates or both. Most preservative suppliers do not test their preservatives against Burkholderia cepacia complex isolates. I would not be surprised to see inhibition in the growth of Burkholderia cepacia complex isolates due to the lack of antimicrobial activity.

Just because the USP AET does not include Butkholderia cepacia ATCC 25416 as one of the challenge test organisms, it does mean that it is correct. I have found that many companies are using this organism as a challenge test organism. As far as using the wild-type isolate of B. lata, I do not have an issue, but there are problems with genetic drift if you are trying to use the isolate within 5-transfers over a long period of time. I have mostly used wild-type isolates from QC microbial product failures to see how robust a products preservative system is effective against the recovered isolate from a test failure.

A4: Historically when the pioneering bacteriologist Robert Koch investigated germicidal activity he failed to neutralize the germicides and overestimated their activity.

If the Phoenix Effect does not exist why do we take the antimicrobial effectiveness out to 14 and 28 days?

A5: Koch was indeed incorrect for not conducting neutralization studies of his germicide studies. In today's environment, Koch would probably get a FDA Warning Letter or be placed on the ban list for his poor experimental design in over estimating germicidal activity. However, Koch did not know better at the time when he conducted his experimental work.

I do believe that the so-called Phoenix Effect in challenge testing is due to both poor neutralization of the antimicrobial activity for preservatives and improperly designed preservative systems in product formulations. Preservatives may have cidal or inhibitory antimicrobial activity against microorganisms. If you have a non-robust preservative system, it is very possible to see a non-reduction with an eventual increase in microbial numbers during challenge testing. With a preservative system that is not chemically stable in a product formulation, it could allow the growth of organisms in a test sample over time especially if the preservative has only inhibitory antimicrobial activity. A preservative example of this is with dehydroacetic acid that only has inhibitory activity against molds. If the preservative is cidal, is the usage concentration in the product formulation above the MIC/MLC levels against a braod spectrum of representative test microorganisms?

I can see of conducting a challenge test out to 14 and 28 days after inoculation especially if the preservative system of a product formulation has only inhibitory antimicrobial activity. With non-sterile product formulations, I do not think that the current USP AET sampling time points is appropriate because consumers will use the product formulation again either that day or the next day. Consumers do not wait for 14 days after initial product usage to use again. In this aspect, I think that a 3-log reduction at either 2-days or 7-days after inoculation would be more representative in how non-sterile product formulations are used with a preservative system that has cidal activity against bacteria. I can see either no increase or a 1-log reduction at 7 or 14-days after inoculation. I do think that more stringent challenge test criteria should be in placed for sterile products because they are often used several time a day if they are packaged in a multi-dosage container (Personal belief)

In general, most pharmaceutical microbiologist or formulators do not have an understanding in how to properly design preservative systems for product formulations. However, there are many books out there by J. Kabara, David Steinberg and Don Orth that explains how to properly design a preservative system. In addition, I have given several presentations on the web that are available on this topic. If preservative system were properly designed so that there is no re-growth of microorganism in a product formulation, we would not be talking about the Phoenix Effect or Period After Opening (PAO) on product labels in Europe.

A6: I don’t think that we failed to neutralize the residual antimicrobial activity...in other words,I believe that the method used for the recovery of the surviving microorganisms was suitable.

Two methods were in fact used - pour plate which was used to test even the 1:100000 sample dilution (even most antibiotics get eliminated at that dilution)...and membrane filtration...filtration was always used for 1 ml and 0.1 ml of the sample - note that the sample is liquid, and I must point out that it consists mainly of water, so it gets through the 0.45 micrometer filter completely.Suitability of all these methods was established more than once - I also had my doubts.

I still do, unfortunately - which is why I asked about the superiority of BCSA medium and B.cepacia complex specific PCR method...do you have any experience in that field?

A7: https://www.pharmaceuticalonline.com/doc/drugconsumer-product-preservation-making-sens-0001 <https://www.pharmaceuticalonline.com/doc/drugconsumer-product-preservation-making-sens-0001>

Still applies to this day.

USP <60> FDA Feedback

As one of the early adopters of the new USP <60> test chapter, I have received possibly the first feedback from FDA about BCC data submitted to the agency since the chapter went effective December 1st, 2019 that I'll share with the group.

The FDA reviewer is under the impression that all five microorganisms listed in USP <60> Table 1 are to be used in method suitability testing. There's the three Burkholderia species, but Table 1 also lists S. aureus and P. aeruginosa which are listed as inhibited microorganisms in USP <60> Table 2.

I believe the reviewer is mistaken and this feedback most likely originates from the header of USP <60> Table 1 which is titled "Test Strains of Microorganisms for Growth Promotion AND Suitability Testing".

Inhibited organisms are not used in suitability testing per the current USP <62> FAQ #12, so I would assume it is not required for USP <60> as well. For example, MacConkey broth is checked for S. aureus inhibition during media qualification, however S. aureus is not used in method suitability of the USP <62> E. coli test. I have contacted the USP liaison for chapter USP <60> (Dr. Radhakrishna Tirumalai) and confirmed the intent of Table 1 is to test BCSA for inhibition of S. aureus and P. aeruginosa during media qualification, not suitability.

In our response to FDA, I have explained our position about not using S. aureus and P. aeruginosa in the BCC method suitability.

If any other early adopters of USP <60> get similar feedback from FDA, I ask that you push back on the request so collectively we can make sure this does not become the industry standard for the BCC test.

A1: Yes, as with any new method people need to become familiar with it. <60> is largely modeled on <62>.

USP <60> states under Indicative Properties:

Suitability of the Test Method The ability of the test to detect Bcc in the presence of the product to be tested must be established. The incubation time for the method suitability should not exceed the shortest incubation period specified. Suitability must be confirmed if there is a change in testing performance or a change in the product that may affect the outcome of the test. For each new product to be tested, perform the sample preparation as described in *Testing of Products*. At the time of mixing, add each test strain in the prescribed growth medium. Inoculate the test strains individually. Use a number of microorganisms equivalent to NMT 100 cfu in the inoculated test preparation. Perform the test as described in *Testing of Products*, using the shortest incubation period prescribed. Bcc microorganisms must be detected with the indication reactions described in *Interpretation*.

As this section specifically refers to Bcc and instructs to use each test strains separately you would use B. cepacia, B. cenocapacia and B. multivorans as instructed in Table 2 for use for both growth-promotion and indicative properties. S. aureus and P. aueruginosa is used to demonstrate the inhibitory properties of the BCS agar.

Mas 100 correction table

Is it necessary to use the correction tables for the Mas 100 results.?
The results are not absolute.
Any justifications for not correcting?

A1: For the MAS-100 NT with the 300 hole head, the Feller correction begins at 18 CFU. Anything under that doesn't have any correction factor. For the 400 hole head the correction begins at 21 CFU. The physics of air sampling with Andersen impaction samplers does require a correction based on statistics.....but it is just a statistical correction, not an absolute CFU count. At 18 CFU, the probability of two colonies landing on top of each other goes up and the colonies can become indistinguishable.

A2: I have never been a fan of the feller correction.

Hard to justify using a mathematical probability formula in an non-homogenous environment on a device that does not have 100% recovery. No sampler can guarantee 100% biological efficiency nor physical efficiency in a cleanroom (sure, some can come close and are better than others).

Unfortunately, microbiology is not a science of mathematics and recoveries of organisms are tedious enough without applying probability to the mix.

Not too mention if you start to get into that “oh so fun” gray area of the Probable counts exceeding your alert or action limits when your actual counts do not. Trending of actual counts will provide more benefits than trending of actual counts (despite the fact they both offer the same data).

Just keep trending your recoveries and use that to demonstrate states of control.

Raw material endotoxin testing

In parental drug manufacturing, does every raw material require bioburden and endotoxin testing even if the vendor doesn’t supply on their CoA?

Wallemia sebis

Has anyone recovered Wallemia sebis from a product containing preservatives? How did it in act in the presence of preservatives?

Has anyone tried to use Wallemia in their disinfectant validation? How did it go?

A1: Wallemia sebi, is considered a moderately xerophilic, non fastidious, slow-growing mold found in food spoilage of low water activity products that has a minimum water activity for growth around 0.70-0.75. At higher water activities it would be outgrown by other molds and bacteria.

Like other molds, antimicrobial preservative systems are fungistatic hence the lower log reduction requirements.

The addition of other challenge organisms to the USP ,51> would be made based on a risk assessment. To me it would be an unlikely choice.

A2: I have experienced many mold circumvent antimicrobial/preservative efficacy.

One of your CMO in the past was a good example.

Remember the fungi we use for antimicrobial/preservative efficacy are easy to kill.

A3: Wallemia sebi is a xerophilic mold that is a common food contaminant that are either highly sugared or salted such as jams, bread, cakes, sugar, bacon, salted meats and salted fish. It is also commonly found as an isolate in indoor air and soil. In addition, Wallemia sebi is also known to grow in products that have a low water activity level. A good reference on this genus is as follows:

Zajc, J and Gunde-Cimerman, N. The Genus Wallemia-From Contamination of Food to Health. Microorganisms. 2018, 6,46.

I believe that the organic acid preservatives such as benzoic and sorbic acid should have antimicrobial activity against this mold species. It is not unusual to use organic acid preservatives to preserve foods against mold proliferation.

Because it is not a common mold isolate in a pharmaceutical environment, I do not think that it would be routinely used by most companies in disinfectant coupon studies. If it is being obtained in your air samples, it could be from the air that is being drawn into a facility in which there is major construction in which the soil has been distributed or farming is being conducted outside of the plant property due to generation of dust aerosols by the wind. However, I believe that a disinfectant that has activity against mold spores would be more than sufficient to take care of Wallemia sebi spores if this species is being isolated in your environmental test samples.

A4: There is a huge difference in the antimicrobial activity of disinfectants that are applied to inanimate surfaces and preservative systems included in products applied to the human body. Fungi and human cells both being eukaryotic cells have common sensitivities to antimicrobial agents.

Media Fill

We are currently changing the HVAC unit of the sterile area. Upon completion of the constructions Media Fill will be performed for 3 runs.

I am wondering do we have to perform 3 runs per vial size or in total? Currently we perform media run in 50ml and 30ml vials so do we need to perform 3 runs for 50ml and 3 runs for 30ml?

A1: Replacing a HEPA is a critical change but nothing changed on your process so this is an interesting question. I'm inclined to believe 3 runs at 50 mLs with the slowest speed will show the room and new HEPA are operating as intended. I would definitely pay close attention to your EM data to ensure the HEPA is working correctly.

A2: Can you please provide us with reference for bracketing the vial size. At our facility we are performing 3 batches of media fill for each container volume.

A3: I believe it is contained in the FDA Aseptic Process Guideline.

A4: As per your mail your facility is brand new one After initial qualification you performed the three + three media fill runs for the minimum and maximum by following the bracketing approach.

After that, because of some other reason you were replaced the HVAC UNIT. that include terminal filters.

It is a critical change in the qualified facility.

After replacement you want to ensure that the facility is regarding grade A ENVIRONMENT. for that you are going to prove with the aseptic process simulation.

As per my understanding no guidance documents mention about the requirement of three medial fill runs for each configuration (minimum three and maximum three).

So by including the minimum and maximum you can take three media fill runs.

Or with risk assessment by including the all interventions with number of times and execussion time one media fill run also justifiable.

A5: You can run the largest vial size with the lowest speed machine and the smallest vial size with the highest speed machine.

This is the bracketing concept in my facility.

USP <60> Bcc. Growth promotion of media

To me the chapter for GPT reads as being applicable to BCSA. however I’m wondering what/if anything is supposed to be performed for the Soybean–Casein Digest Broth that you add your product to at the start of the test?

I have come across a USP commentary in response to the draft Bcc chapter (June 2019) where they addressed peoples comments and i saw the following.

Comment: The commenter noted that there is no mention of the growth promotion for the primary media SCDB. This media would be growth promotion tested as outlined in <61>. However , if this media is used for Bcc testing, should Bcc organisms be included?

Response: Comment was not incorporated into the chapter for clarification. Any other media or dilution buffer used for that test should be either growth promotion tested by using the indicated organisms in Table, or it should be tested for lack of inhibition or toxicity by default.

Do you follow <61> and use S.aureus, P.aeruginosa and B.subtilis only or are you expected to also include Bcc strains?
In a similar situation, if you are testing following <62> for some specified organisms e.g. Salmonella and E.coli should you also add these organisms to the standard GPT for Soybean–Casein Digest Broth or are the three defined organisms on <61> all that is required.

A1: If you are going to use Soybean-Casein Digest Medium to enrich for Burkholderia cepacia complex species that are listed in Chapter 61, I would recommend that the 3 Burkholderia cepacia complex species indicated for performing growth promotion of BCSA also be used to verify growth promotion of this microbial enrichment broth. If Soybean-Casein Digest Medium is also going to be used for Chapter 62 testing, I would also recommend the usage of those growth organisms of Chapter 62 in addition to the Burkholderia cepacia complex organisms for growth promotion.

It is highly likely in your laboratory that Soybean-Casein Digest Medium will be used for enrichment of test samples that need to be tested by both Chapters 60 and 62. It just adds to confusion if you separate out lots of Soybean-Casein Digest Medium for usage in only testing for Chapter 60 or Chapter 62.

A2: Would you expect all organisms you are looking for in total count and enrichment routes to be included in the panel of GPT organisms for the broth used just in method suitability testing or also in routine testing? This could become a long list if you test for several specified organisms and also identify some additional organisms of concern for the product.

As the method suitability itself (comparable counts or isolation on selective agar) would show that the low level inoculum of each organism has successfully been grown during the method step. Is that not satisfactory proof of organism capability?

If as a minimum the SCDB is GPT with all of the organisms initially do you think it acceptable to only then use the selected few recommended within <61>. I saw them as selective few chosen that had been identified to represent sufficient organisms for the general purpose broth.

If a neutraliser was added to the broth, in order to confirm no inhibitory impact would you also recommend all organisms to again be included.

anaerobes in non sterile product

is there any information on if and when you need to test non-sterile product for anaerobic organisms?

If a product is manufactured under a nitrogen blanket due to sensitivity to oxygen/atmosphere should testing for anaerobic bacteria be performed in finished product?

What if the product was packaged in final container in absence of oxygen or was not packaged in final container in absence of oxygen but it was just required in manufacturing process?

A1: USP <62> contains a test for Clostridium species.

A2: In general, non-sterile finished products are not tested for the presence of anaerobic bacteria. In some cases, a non-sterile finished product may contain a raw ingredient such as an earth derived raw ingredient such as talc or clay which may contain anaerobic bacteria. Instead of testing the finished

product for the presence of anaerobic bacteria, the earth derived raw ingredient itself is tested for the presence of anaerobic bacteria such as Clostridia species before usage in manufacturing. In addition, I believe that there is a USP monograph for Honey in which there is a requirement for the testing of Clostridia species if the ingredient was going to be in a product for children under the age of 3. In most cases, I believe that companies will test the Honey by itself for the presence of absence of Clostridria before usage in manufacturing instead of the finished product.

When a nitrogen blanket is used to overlay the finished product in a package during filling, the purpose is to prevent exposure of the product to air that could cause chemical degradation of the product before the package is open by the consumer. After the package container has been open, the nitrogen layer is gone and the product is now exposed to air. With nitrogen overlaying during filling, I have found that most companies will not conduct anaerobic testing of the finished product, but will have the nitrogen gas to pass thru a sterile 0.22 or 0.45-micron filter to remove the presence of any organisms that may be present in the gas before usage in filling.

A3: While there is no specific requirement in USP 1111 for absence of Clostridia, we always fall back on “risk-based” thinking. The story that stands out the most for this situation (to me) is the Bacillus contamination with the alcohol pads. No document says Bacillus is an adulterant or that it needs to be excluded for but it did contaminate those pads and there was a death. We don’t need to go down the “well I can’t look for everything” path because nobody expects that, but you should be able to make a scientific argument for or against testing for anaerobes in your product. Patient population, product administration, manufacturing process, and shelf-life (among others) should all be part of your risk-based assessment.

A4: We package some of our components under nitrogen, but we've never been challenged but for maybe once over 3 decades. It was one customer who wanted us to check a few batches, but not a routine thing.

We also manufacture animal tissue-based devices where we definitely check for anaerobes routinely, at least at the raw material stage. Depending on the presence/absence of anaerobes in the raw material and the downstream processing, we may or may not look for them in the final product as well. Anything natural, plant-based etc., it's expected you check. If you're just extruding polymer and packaging under nitrogen, for instance, I wouldn't bother.

Disinfectant test question

We prepare different disinfectant solution to use in sterile area by filtering through 0.2 micron filter under grade A environment.

Should we perform sterility test or bio-burden test for filtered disinfectant solution.

If we have performed disinfectant efficacy and hold time weather it is necessary to perform sterility test or bio-burden test?

A1: The entire manufacturing and sterile filtration process should be validated. For example pre and post filter validation (product specific bubble point). Validated sterilisation of equipment and final disinfectant packaging.

Also you should be looking for a sterility test on the disinfectant solution (but it should be neutralised prior to testing).

Do not draw filtered disinfectant solutions from bulk over several days (disinfectants should be sterile prior to use). There is a risk bulk solutions could become contaminated.

If you fill into sterile single use containers eg trigger sprays (used over several days) use bag in bottle systems. You will need an in use shelf life for this.

A2: Disinfectants used in Grade A critical environments should be sterile and as such should be tested for sterility using a suitable method demonstrated to neutralize the disinfectant. This is not a release test, but rather to provide assurance that your sterile disinfectants are are not contaminated and do not become contaminated during use. My understanding is that it is not necessary to test all batches of prepared disinfectant, but to evaluate the process for preparation of sterile disinfectants on an on-going but periodic basis. Other Forum thoughts?

Regarding industrial practice - Nitrogen & Compressed air

We are using Nitrogen and compressed air in aseptic facility where we are performing sterility test of Nitrogen and compressed air from all the user point daily on schedule base irrespective of direct contact of product or not.

Please comment, what is the industrial practice? Whether to perform sterility test or Bio-burden?

A1: Why?

Compressed gases may have a very low bioburden but they are not a sterile product.

You should periodically use environmental monitoring techniques, e.g., an adaptor to an active air sampler, to monitor a cubic meter of gas. Use sterilizing-grade filers at the point of us that are subject to integrity testing.

Requirement of Nitrogen generation plant

We are planning to introduce Nitrogen generation Plant for our aseptic facility of SVP and LVP product. Plant available in market is producing Nitrogen having purity max 98.5 % and 1.5% Oxygen as per USP. As our few product is sensitive to oxygen can we use above for our facility? At present we are using Nitrogen 99.9 % purity in cylinder form. What is standard requirement for Nitrogen Plant with regard to Purity?

A1: The change would be subject to a formal change control procedure and would used to be supported by stability testing.

A2: That is correct, but the thing is almost all regularly agencies around the world wants to move towards more quality products. It is always hard to get this type changes done.

Microbiological aspects of Product Development

Can anyone point me in the direction of any guidance or list some steps on what needs to be considered or performed associated with microbial quality/testing during the development of a new product (nonsterile, preserved and aqueus).

During formulation (e.g where ingredients and any preservative concentrations could change). Im assuming PET on various formulations under consideration for shelf life studies?

When the final formula has been determined.

Is there more than just performing method suitability on the final product and batches on stability?

A1: You could read my recent book chapter:

Cundell, T. 2020 Chapter 2 - Microbial Contamination Risk Assessment in Non-sterile Drug Product Manufacturing and Risk Mitigation in *Pharmaceutical Microbiological Quality Assurance and Control – A Practical Guide for Non-Sterile Manufacturing*. D. Roesti and M. Goverde (editors) J. Wiley & Sons

Furthermore if you purchase the book you will have access to much excellent material by experience authors.

A2: In general, there is no formal guidance concerning the microbiological aspects of a product during development. Most people will perform a risk assessment to determine what particular aspects of microbial testing needs to be performed during development. From my experience, microbial testing performed during product development will vary from company to company, but there are several common steps between companies and they are as follows:

In general, you would conduct a microbiological risk assessment to determine which of the new raw ingredients can be susceptible to microbial contamination and perform microbial content test methods on those raw ingredients that are susceptible to microbial contamination to ensure that they are of an acceptable microbial quality. Susceptibility is conducted on those raw ingredients before analysis is conducted on it to ensure recovery of microorganisms.

For proposed new finished non-sterile product formulations, microbial content treating is performed after conducting susceptibility testing to ensure that you are able to recovered microorganisms from a test sample if the formulation had been determined to be susceptible to microbial contamination during manufacturing.

During product development, it is not unusual to test several variations of the non-sterile product formulation for preservative adequacy to ensure that the product does not contain raw ingredients or has experienced processing steps that can cause inactivation of the preservative system. At this stage, it is not unusual to see a formulation to be challenge tested with additional tested organisms than what is listed in the USP AET Chapter and an increased sampling frequency from only conducting sampling 7, 24, and 38 days after inoculation to determine preservative adequacy with more stringent log reductions.
The purpose of this step is to ensure that a robust preservative system is present in a formulation to protect it during consumer usage.

After a formula has been selected , it is not unusual to test formula in different packaging types to determine whether the preservative system is stable over time by using accelerated aging conditions and the composition of the packaging did not have an adverse effect of the preservative system by either causing absorption, inactivation or chemical decomposition of the preservative system. I have also seen companies to perform microbial in use studies of packaged test samples that have been used by consumer panelists to see what type of microbial bioburden is present in a formulation after usage and to see how well the preservative system behave during product usage.

After accelerated testing, it is not unusual for people to check on the levels of preservatives in a product formulation by doing chemical analysis.

A3: I'd like to add to Don's product development list that ICH Q6A has guidance which says you should demonstrate your lowest specified concentration of preservative content will pass AET.

For example, if the proposed specification of preservative content on assay is 80-100% throughout shelf life, typically a firm will purposely make a batch with 80% preservative content and test it per USP <51>. If it passes, you have data to support the preservative system should work even if the preservative degrades to that lower level during shelf-life. If it does not pass, you need to consider tightening the preservative content specification and/or consider a shorter shelf life.

You don't want to find out your preservative system has stopped working after your product has been on the market for a few years.

A4: [name redacted], yes demonstrating that the multi-use product passes the AET at the lower shelf specification of say 80% of the label claim is a good strategy and provides evidence to default to chemical assay for the preservative level in stability studies.

However, in my career I did encounter a national board of health that asserted that as the preservative worked at 80% of label claim it should be reduced further. Go figure!

A5: I would add that the 80% preservative content may be a predictor of end of shelf life performance; however, this would need to be proven through stability studies, as some aged formulations can exhibit a different antimicrobial profile than freshly made batches with a lower preservative level.

A6: The stability requirement is at least one batch preferably three are taken out to expiration at the labeled storage conditions as the preservative may be bound to an ingredient and be ineffective.

A7: I have first-hand experience that a freshly-made batch at 80% of label claim did not perform the same (AET results) as room temperature stability samples that reached 80% of label claim at the end of shelf life. For this reason, the chemical assay for stability samples did not correlate with the expected AET results.

As such, a lab batch made at 80% may only <predict> end of shelf life AET results, but this still needs to be confirmed.

A8: I've seen this as well. Even accelerated stability conditions cannot always predict how the product will hold up at end of shelf life at room temp. Another quite frustrating thing I see occasionally is preservative content that still comes in at 100% on stability assay, but it doesn't pass AET even though it was working at T=0.

A9: During product development, it is unusual to place 3 separate batches of a product formulation up in a real-time stability testing program unless there is a need for additional material for clinical studies. During development, most challenge testing is performed on freshly prepared and artificial accelerated aged samples.

When a formulation starts production in a manufacturing facility, it is common practice to take 3 separate production batches and place them into a real-time stability program. However, I have seen many times that there is not a sufficient demand for a product formulation in which there is only 1 production batch that is manufactured.

In regards in making a fresh product formulation that contains 80% of the preservative level, I believe that it is a common practice at some companies to determine a minimum preservative level In order to validate a preservative level in order that microbial challenge testing does not have to be performed during stability testing. As Michael had indicated, I have also seen the same issue in preservative adequacy of the formulation in a freshly prepared batch with 80% preservative level and real-time aged stability samples.

Instead, I would conducted accelerated aged studies of a formulation during development in which challenge testing and chemical analysis would be conducted. For real-time stability samples, challenge testing would be conducted and the analysis of the preservative levels would also be done at yearly time points. I would not set a specification for a preservative level in a product formulation until real-time stability testing has been completed at the end of shelf life in which there is both passing challenge test results and a level of degraded preservatives that are present in a formulation.

Using a freshly prepared batch of a formulation containing 80% of the preservative system is not truly representative of other chemical and physical changes (e.g pH and viscosity changes) that could occur in a product formulation during real-time stability aging that may have an adverse effect on the preservative adequacy of a product formulation. Preservative adequacy of a formulation is not just above preservative levels.

A10: I agree with you that sometimes that the preservative challenge tests from an accelerated aged sample does not predict to what happens for a product formulation during real-time stability testing. Part of the problem is that some people during development will do accelerated aging of a bulk formulation and not conducted accelerated aging of the product in the proposed package. Over time, packaging can have an adverse effect on the preservative adequacy of the formulation.

In regards to your issue in which there is 100% of the preservative and the formulation is found to be inadequately preserved at latter time points in a stability study, it is common for some preservatives to bind to certain raw ingredients of a product formulation and the preservative is no longer available to provide antimicrobial activity to the formulation. I have also seen some preservatives to become enclosed in micelles and they are no longer available to provide antimicrobial activity in a product formulation. A perfect example of this phenomena is Caprylyl glycol and surfactants.

When using preservatives in a product formulation, there is a lot of chemistry that microbiologists and product development chemists are unaware of in making a product formulation to be adequately preserved. Sometimes it is an art than a science in developing an adequately preserved product formulation from my experience. It is also important for microbiologists to have an understanding of the chemistry for preservatives in how they work or do not work than just knowing how to perform the challenge test.

Utilities used in pharma - compressed gas and purified water

i am used to monitoring water and compressed gas (Air and Nitrogen) systems where it is generated and distributed on site and you build in a routine monitoring program from locations.

However, what type of qualification and monitoring is required where these are purchased (compressed Nitrogen in cylinders and purified water (DI) in bulk containers) for use if they are needed on a smaller scale and no existing supply from the main site distributions?

Accept CofA, test each cylinder/bulk water container before use, random sampling of cylinder/bulk water container?

A1: At our facility we don't have a nitrogen generation system, we are buying cylinders from outside vendor.

Our checklist includes:
Vendor approval
Vendor COA
Testing of each lot received (normally the first cylinder)
Periodic testing from user point as per testing schedule or matrix.

We perform system qualification every 2 years.

Mike, i do have a question for you:
We are planning to introduce nitrogen generation system on site but most of the vendors provide the system with 98% generation purity specifications as per USP. Currently the outside vendor is providing us with 99.97% purity of nitrogen. Can you suggest what do we do? We have a sterile aseptic processing parenteral facility. Our products are highly susceptible to Oxygen.

Burkholderia cepacia - A critical germ for Environmental Monitoring

Can you please give advise for Burkholderia cepacia as critical germ for Environmental Monitoring? We identify all findings on microbiological EM samples by Maldi-TOF and would react for exceedings of warning limit or if objectionable organism. Is it necessary to define Bcc as an objectionable ?

Additional information: - production of oral solid dosage forms - cleanroom class D.

A1: The term objectionable microorganisms is related to non-sterile products. No microorganism is objectionable until it is found in a product.

Bcc is more likely to be found in purified water and if it is used as ingredient water for an aqueous non-sterile product this would be a concern.

Bcc is less likely to be found in air, surface or personnel monitoring but it could persist on processing equipment incompletely cleaned and stored wet.

You could refer to my 2019 publication:

Cundell, T. Excluding Burkholderia cepacia complex from aqueous, non-sterile drug products Amer. Pharm. Rev. 22(1): 36-41 2019

A2: BCC (B. cepacia complex) is a required organism for release of non-sterile aqueous products. If you make that type of product, you may choose to evaluate if it is in your environment, but alone it is not a critical EM organism.

A3: [name redacted] is absolutely correct that the term "objectionable microorganism" should only be used for microbial isolates that had been recovered from non-sterile finished products.

From microbial air sampling, I have also seen the detection of Candida albicans, Aspergillus braselienesis and Aspergillus fumigatus. If Candida albicans and Aspergillus brasilensis had been detected in a finished product, it would be rejected. If Aspergillus fumigatus had been isolated in a finished product, I would consider it to be objectionable.

It is not an unusual occurrence to isolate potential finished product isolates during environmental monitoring that would be considered as objectionable if present in a non-sterile finished product formulation. By identifying organisms that have been recovered from conducting environmental monitoring, it aids in determining the root cause of a microbial product rejection for the presence of a particular microbial species.

In general, Burkholderia cepacia complex species can be isolated in water samples from either a purified water system or tap water that is used in the cleaning of manufacturing equipment or the facility itself. If you are detecting Burkholderia cepacia complex species by surface monitoring after conducting sanitization, it means that the equipment is still wet and water is present somewhere in the equipment. In addition, the effectiveness of your cleaning and sanitization procedure of manufacturing equipment are suspect.

I have seen personally the isolation of fluorescent Pseudomonas species (e.g. Ps. aeruginosa, Ps. putida, and Ps. fluorescens) and Burkholderia cepacia complex isolates in microbial air samples. The reason for the isolation of these Gram-negative bacteria species in microbial air samples was due to the presence of generated water aerosols that had been formed either during the addition of deionized water to mixing tanks or from the cleaning of manufacturing equipment or facility surfaces in which tap water is being used. The reason for isolation was due to microbial air sampling was also being conducted at the same time as these 2 activities were being conducted.

If you are isolating microbial isolates that could be considered as objectionable in a non-sterile finished product formulation during environmental monitoring, there is always a reason.

A4: As you have oral solid dosage forms manufacturing, you need not to include burkholderia as objectionable microorganisms.

You can justify it by referring to USP general chapter scope.

Also if you are getting burkholderia in environment monitoring program i would suggest you perform a risk assessment.

You can use data obtained from - water activity, MVTR test, PET etc for risk assessment.

neutralizing carbamide peroxide

Wondering if anyone has a method to neutralize carbamide peroxide? We have a formula using this compound at 7.2% and need to neutralize for suitability testing purposes.

A1: Hi Caron. If it is insoluble in water, I would use warm IPM then fluid K rinse, and fluid A as final rinse.

A2: It is my understanding that Carbamide peroxide is urea hydrogen peroxide. According to the MSDS for this material, it is a crystalline solid that is easy soluble in cold water. You do not need IPM to dissolve it if it is incorporated into a product formulation. To neutralize the antimicrobial activity of this raw ingredient that is present in a product formulation, I would recommend the usage of Catalase. I have seen people use a 0.01% Catalase solution to neutralize hydrogen peroxide. Depending upon your suitability test results, you may need to increase the concentration of Catalase in your diluent to neutralize hydrogen peroxide concentration that is present in your formulation. You also need to remember that a Catalase diluent cannot be sterilized by using an autoclave because heat will destroy the enzyme. A stock solution of Catalase will need to be filter sterilized and a sterile aliquot will need to be added to the diluent.

A3: We did consider this and we have ordered catalase. We also ordered urease because the urea will also need to be neutralized, will it not?

A4: I have heard that acryl urea derivatives will have antimicrobial activity, but urea by itself has no antimicrobial activity. Your suitability testing with and without urease will indicate as to whether you will to use the combination of urease and catalase.

A5: Urease would hydrolyze urea to ammonia which is more toxic.

at rest environmental qualification

I am going to perform environmental monitoring performance qualification for an area that is already in use. The room is in intensive use, 24/7. In these circumstances what is the need for the "at rest" stage of qualification ? How would i perform "at rest" monitoring?

A1: When did the term EMPQ arrive on the scene? As I see it it is the environmental controls that are being qualified not the environmental monitoring.

A2: If area is in use then we have to perform area requalification.

Normally the difference between at rest and in operation state is limits, if in operation limits are less then at at rest then you don't have any problem.

Conduct 3-3 days qualification.

A3: At rest monitoring is generally used to demonstrate that the area is operating within its design requirements and is done prior to releasing the area for manufacturing use.

With an area already in use, there is no good reason to perform at rest monitoring. If, at some point in the future, the area is taken out of service for modifications, at rest monitoring should be performed prior to release back to manufacturing.

A4: I think a part of it is the obsession with acronyms, to reduce the wording. Texting culture hasn't helped.

Question about environment monitoring using Biocapt

As we all know that one of reputed company `*particle measuring system`* have various range of excellent products. The product name as Biocapt which i found good for reducing the cross contamination during environment monitoring.

So, my question are:

Can i use Biocapt for environment monitoring in clean rooms of pharma industry?

Does biocapt comply with US-FDA norm?

How can i overcome if want to do visual inspection before start sampling?

A1: I'll assume you are referring to the single-use device? Yes you can use the BioCapt, in conjunction with any of their samplers designed to utilize that disposable sampling head in a pharmaceutical cleanroom.

Yes, the BioCapt device, when utilized properly, will allow you to generate data that supports a risk based and justifiable EM program. That would then be in compliance with the "FDA norm" (which does NOT specify air samplers).

Are you referring to visual inspection of the BioCapt (checking for obstruction) or are you referring to the agar plate itself, ensuring no prior contamination before use? Either way, you just simply need to look at the plate and confirm there are no issues. Not sure what issue there is to overcome.

Garment Particle Testing per IEST-RP-CC003.4 - Helmke Drum Test

I have a question regarding garment particle testing per IEST-RP-CC003.4 using the Helmke Drum Test.

What is the expectation of where this test is performed with a bench top drum? Can this test be performed in a laboratory setting (non-classified room) in a ISO 5 LAF hood or is it expectation that this test is performed in a ISO 5 classified environment? What is the rationale for your chosen environment?

R2A Plate Media Incubation Guidance for Optimal Recovery of BCC from Water

I am seeking additional references/clarification for incubation temp and time for optimal recoveries of BCC orgs from manufacturing bulk water samples (if present) when using R2A plate media in conjunction with membrane filtration only. EP monograph for Water, Purified .0008 describes a minimal incubation time of 3 days at 30-35C whereas guidance from Standard Method for Examination of water/wastewater 9213E recommends incubation at 35C for minimum 48 h but with option for 20-28C for 5-7 days for optimal recovery (i.e. highest quantitative counts for HPCs in general). Which of these two options should be applied for use in recovery specifically for BCC orgs?

A1: See Cundell, A. M. Water Monitoring *in* Microbial Contamination Control in the Pharmaceutical Industry Edited L. Jimenez Marcel Dekker 2004.

A key reference would be Reasoner, D.J. Monitoring heterotrophic bacteria in potable waterIn: McFeters G.A. editor. Drinking Water Microbiology: Progress and Recent Developments. Springer-Verlag 1990 pp452-477

In general, the less rich the media, the lower the incubation temperature, and the longer the incubation time the higher the count. In terms of methods spread plate > membrane filtration > pour plate.

For a monitoring program designed to detect adverse trend, choice a method and stick with it. Like most microbiology, no absolutes here.

A2: You could always try to use a version of the JP General Information, G8 Water Quality Control of Water for Pharmaceutical Use under the R2A growth promotion testing. You would be using the Bcc cultures instead of the M. extorquens and P. fluorescens cultures:

4.4.2. Media Growth Promotion Test
In the media growth promotion test with the R2A Agar
Medium, use the strains listed below or other strains considered equivalent to these strains. Prior to the test, inoculate
these strains into sterile purified water and starve them at
20 - 25C for 3 days.
Methylobacterium extorquens: NBRC 15911
Pseudomonas fluorescens: NBRC 15842, ATCC 17386,
etc.
Dilute the fluid containing the strain starved with sterile
purified water to prepare a fluid containing about 5 × 101 -
2 × 102 CFU/mL of viable counts. When pipetting 1 mL of
the diluted fluid onto the R2A Agar Medium and incubating
at 20 - 25C for 4 - 7 days, sufficient proliferation of the inoculated strain must be observed.

I would test them at both 20-25C and 30-35C and do a comparative study on best recoveries.

A3: In addition to Tony Cundell's work, Tim Sandle did work using R3A less nutrient media for pharmaceutical water. It stated: "the R3A recovered B. cepacia satisfactorily"

https://www.researchgate.net/publication/267332592_Assessment_of_the_suitability_of_R3A_agar_for_the_subculture_of_microorganisms_isolated_from_pharmaceutical_water_systems/link/544bfca70cf24b5d6c409234/download

A4: For optimal recovery R2A is best to incubate at 30-35C for 5-7 days

FDA Compounding Guideline

I recommend that you read and comment on the FDA cGMP Guidance for Human Drug Compounding 503B Outsourcing Facilities, Revision 2. January 2020.

A1: USP-NF also posted this today, announcing Standards Affected by Postponements of revisions of several USP chapters: https://www.uspnf.com/notices/795-797-postponement-ref-changes. Subscription to USP-NF is free.

Reduce in water sampling and testing frequency

I am working in pharmaceutical manufacturing industry, I want to reduce water sampling and testing frequency. Is there any literature or any guideline available which I can refer to reduce the frequency. And what are the essential steps I need to take care while reducing the sampling frequency of water.

A1: A reduction in frequency will be based on a risk assessment. Your historical test data, current sampling plan, rotational schedule, sanitization schedule and type of water system will help to determine whether a reduction in sampling and testing is acceptable.

A2: I would suggest to refer on the historical data of the water system within an risk analysis. In relation to maintenance work and perhaps online monitoring of some parameters, it may be possible to reduce the testing frequency.

A possible way could be: Every week only a quarter of all sampling points (spread over the whole system) will be sampled (+ return flow/s + feed water). So every four weeks every sampling point is measured. Therefore historical data has to document, that every sampling point is representative for the good quality of the water in the loops. Normally this should be already shown with the qualification data (PQ-phase I, II & III) of the system.

If you do not have historical and qualification data to verify the consistent good state of your system, I would not suggest to reduce the testing frequency. Please consider, that water is one of the most critical aspects in pharmacy.

Clean room air samplers air flow rate

I would like to know is there any guidelines mentioned air samplers airflow rates?

Most of them are following 100 lit/min and 180 lit/min air flow rates which guidelines is saying to follow these air sampling

If I follow 200 lit/ min air sampling (5 mins) to save the time and work load Is it acceptable 200 lit/min air sampling in pharmaceutical clean rooms ?

Please share any document references for airflow rate for air samplers.

A1: There are a couple things to think about with viable samplers. First, ISO 14698 is an international standard that talks about impaction speed, physical and biological efficiency and unidirectional airflow. As an example, you could have a sampler that ran at 1000 L/min but the biological efficiency of recovering organisms would be very low. You need a sampler that gives you good biological efficiency as well as the physical efficiency to pull particles in down to about a micron so there is a balance between pulling in the particles that organisms can be found on, and the ability of those bugs to grow once they hit the media. Physical and biological efficiency are the two main drivers behind how fast you can run an active air sampler. There is also the unidirectional flow rate that can disturb laminar flow. If you perform a smoke test with your sampler and notice turbulent air around the exhaust due to a higher flow, that could lead to pockets of air that don’t get filtered. The smoke test can be done by the manufacturer or a third party. The physical and biological efficiency should is usually done by a 3rd party to confirm the manufacturers claims.

A2: There is no guidance for which flow rate to utilize.

However, you must ensure that the sampler does not create turbulence not generate particles and can be properly disinfected. Placement of the units is therefore critical (I do not condone the use of a portable sampler in an ISO 5 environment).

At 200 L/min (5 minutes time) that as an awfully small snapshot of time to detect contamination or deterioration of the environment. Be prepared to take more samples.

You’re free to use whatever sampler you want, however, be sure justify and rationalize it’s use in your EM program. The manufacturer should provide you the physical and biological efficiency of the sampler (ISO 14698 validation) to support your justification.

Biological Indicators

For Biological Indicators used in steam sterilization studies is there an F Bio requirement for BI selection using the overkill method?

A1: This is clearly explained in PDA Technical Report Number 1 (Revised).

They claim Fbio *>* 12 minutes

A2: We have recently discovered growth on Cetrimide Agar plate for our PW testing (Detection of Pseudomonas aeruginosa) . We sent the recovery out for identification and the organisms ID were Brevibacillus choshinensis (GPB) Staphylococcus vitulinus (GPC) and Kocuria rhizophila (GPC). To my understanding Cetrimide Agar can only grow GNR organism because it is highly selective. Also, note that the testing method performed was membrane filtration and these filters are also sterile. Has anyone ever encountered something similar to this and may share an explanation as to why?

A3: I have also experienced this issue when selective/differential agars such as Cetrimide Agar, MacConkey Agar, VJA, Mannitol Salt Agar and Baird Parker Agar was not 100% selective for isolating the target organism. I have found that selective/differential agars are not 100% inhibitory to prevent the isolation of non-targeted organisms. It is common to isolate Gam-positive bacilli and other Gram-negative bacterial species on these agars because they have the ability to be not affected by the inhibitory ingredients that are present in these microbial media.

A4: We have seen this with membrane filtration using Pseudomonas isolation agar (PIA) when testing WIP water samples. We have recovered other genus this way that were subsequently unable to grow when subcultured directly to the same batch of PIA. It seems that the membrane was limiting the Irgasan™ component from fully inhibiting their growth. It's become part of our standard process now to subculture this way before counting anything from that test as a Pseudomonad.

Microbial Testing for Drug Delivery Implants

Can you provide guidance for microbiological sample preparation and testing on solid non-biodegradable extruded polymer passive drug delivery implants? Specifically, for pre-sterilization bioburden testing and sterility testing, is there an expectation to test just the outside surface of the implant, like one would for a medical device, or to test the "inside" of the implant since the polymer matrix elutes drug over time?

USP <1> implies that the implant may be dissolved but acknowledges that the solvents and conditions required to do this would be microbiocidal. ANSI/AAMI/ISO 11737-1 gives helpful guidance, but seems to deal only with the outside surfaces. There are a number of non-biodegradable polymer implants on the market used for contraceptive drug delivery; does anyone know how those are tested?

FW: Annex 1- Gr B cont viable monitoring

The Annex 1 draft has verbiage indicating continuous viable monitoring should be performed in Grade A and B for the full duration of critical processing. This seems to suggest that Grade B should be continuously monitored for viables.

How is this being interpreted? Is this something you will be implementing or will you be writing a risk assessment to justify why continuous viable monitoring in Grade B is not needed?

A1: Do you mean to use the word verbiage? I encounter it when people probably mean text or wording.

Verbiage is defined as an overabundance or superfluity of words, as in writing or speech; wordiness; verbosity. manner or style of expressing something in words.

I believe the EU regulators mean settle plates when they refer to continuous viable monitoring.

A2: Sorry I do not have an answer for you but I do have a follow up question... as plates dry out quickly under active viable monitoring, is continuous monitoring required during the entire fill using both active air monitoring and settle plates?

A3: In my company we are doing continuous monitoring in grade A and grade B area surrounding the filling line. So all samples in the aseptic filling room are continuously exposed for passive air sampling during set-up and filling.

A4: How often are you doing active viable sampling?

A5: Active air at the beginning, middle and end of the batch, and after critical interventions. But passive continuously.

Incubator dedicated

It is right to incubate in different incubator the sterility test samples and the growth promotion test?, it is stablished on a GMP document?

gloves nuclear pharmacy

A friend is performing glove qualification for a nuclear pharmacy. The room is Class C with a Biological Safety Cabinet of Class A.

What should be the limit for the gloves?

No CFU on gloves (Class A)
or
3 CFU for gloves (Class A in Class C)

With a justification that the technician is in a Class C room.

Any other alternatives?

water system validation

I have some doubts on water system validation 1. What is difference between phase 1 and 2 2. How to know how many days phase 1 and phase 2 (any reason to selection of number of days) 3. Any gap Between phase 1 and 2 (because Mlt analysis takes 6days) or continuously perform phase 2 after phase 1 4. Any gap Between phase 2 and 3 (because Mlt analysis takes 6days) or continuously perform phase 3 after phase 2 5. Where is alert and action limit establish (phase 1 or 2 or 3) 6. Can we use produced water from water system After phase 1 (phase 2 analysis is running) 7. If we establish alert and action limits in phase 2 any deviation occur (cross the alert and action different seasons) in the phase 3 how we explain that one. 8. If any deviation occur after phase 3 in alert and action limits any changes required in alert and action limits 9. How to establish sanitization frequency.

Confirming D-Value on Purchased BIs

I am currently performing USP population count assessment on incoming steam BIs and confirming manufacturer's D-value although the D-value confirmation is not required by USP as acceptance testing for steam sterilization BIs. I am looking to determine routine practices for other users?

Do you perform D=value confirmation testing? If so, on what interval?

Microbiological testing of raw materials and components

What is the expectation on the micro testing of these if used in pharmaceutical dosage forms, either steile or non-sterile?
Is it a must do, recommended to do or no guidance and at your discretion.

Any experience or actual references to industry practice or more ideally regulatory documentation would be appreciated.

Class A hood in Class C room

To generalize from my previous question. If i have a Class A laminar flow hood or Biological Safety Cabinet, situated in a Class C or less room, what limits should the gloves be ? I believe that even with the best operator there is an influence of the surrounding Class C environment. Is there any regulator guides for this situation?

A1: Why would a Grade A hood be in a Grade C (or below?) room? If you're doing anything critical at all in that hood, it's generally a good idea to keep the surrounding area clean enough to not make the operators gloves inside the Grade A hood soiled. There should be no recoveries on the gloves when performing critical tasks in a Grade A hood.

A2: If you want a guidance for limits EU annex 1 is a guide you may refer to. You may also reference USP 1116, FDA: Guidance for Industry- Sterile Drug Products by Aseptic Processing cGMP and PIC's Guide to Good Manufacturing Practice for Medicinal Product Annexes.

What is being done in the hood? Sterility testing, Aseptic filling non-sterile manufacturing? Also application of limits for certain countries should also be reviewed.

Your process that you set your specs for (alert/Action levels) should be developed through a Risk based approach. Based on your risk assessment of your process you develop the adequate controls such as cleaning, PPE, Aseptic techniques, smoke studies, environmental monitoring which you would apply to decision for the classification of room (background) and the critical area (Grade A flow hood). Then based on classification and process you could develop your limits (usually done through an environmental performance qualification).

It just not about applying numbers based off a guidance. It also requires some scientific examination to your process with scientific documentation for application of the limits you will apply.

-----Original Message-----
From: The Pharmaceutical Microbiology Forum Email List
<PMFLIST@LISTS.MICROBIOLOGYNETWORK.COM> On Behalf Of Joel Russo
Sent: Wednesday, February 19, 2020 5:13 AM
To: PMFLIST@LISTS.MICROBIOLOGYNETWORK.COM
Subject: Re: [PMFLIST] Class A hood in Class C room

Why would a Grade A hood be in a Grade C (or below?) room? If you're doing anything critical at all in that hood, it's generally a good idea to keep the surrounding area clean enough to not make the operators gloves inside the Grade A hood soiled. There should be no recoveries on the gloves when performing critical tasks in a Grade A hood.

A3: The answer to the query is it depends on what activities are being performed in the Grade A hood. For example, microbiology labs often use a Class II A biological safety cabinets to provide a zone of local protection to protect samples and tests from extraneous contamination while the surrounding lab is either not classified or Grade D. A similar scenario may be used for zones of local protection during formulation or less critical manufacturing activities that are designed to minimize bioburden prior to aseptic filling or

manufacture of products that will be terminally sterilized. Regulatory requirements are fairly clear on critical activities but less so on other activities. For the less critical activities you should assign microbial limits based on risk to the operation and review of actual data, i.e. process capabilities.

A4: It is nuclear pharmacy. The guides for making injections for immediate use allow Class hood in Class C room. My question remains , can i justify having glove limits lower than Class A because of the surrounding room.

A5: Just to add to this. For cellular therapy aseptic processes, BSCs are employed either Grade A in Grade B background or ISO 5 in ISO 7 background. Due to the manual nature of processing, that many BSC ops can be way upstream of final product, and that operators may handle with their gloves a wide variety of lab materials / components that are not rendered "sterile" in their outer packaging, to require no recovery on gloves for these type of processes may not always be scientifically sound or practical.

A6: Glove counts must adhere to the classification level that they will operated in.

There is a miscommunication in this request. [name redacted] needs to be more clear under what regulation or guidance he is working in.

If he is operating under USP<797> or pending USP <825> and if this is limited to *Immediate Use* then glove testing and surface sampling is not required.

However if any higher risk level aseptic processing occurs than the applicable glove limits for applicable required class requirements must be applied.

Regarding GPT

I have one query regarding GPT

We perform GPT of R2A agar Medium and in that medium we also performed Water isolate.

So my question is, That water isolate is Gram Positive or Gram Negative?

Because only Gram Negative Bacteria exist in Water system Acc. To USP.

So only Gram Negative Bacteria will be use as in-house isolate,,but we also use Gram positive bacteria in R2A Agar example- Bacillus, so what is the main logic for selecting Water In-house Isolate whether it is Gram positive or Gram Negative?

A1: Better to Use Gram Negative isolate for R2A Growth Promotion Test.

Thermophilic spores positive plates images

Can anybody send positive plates of Thermophilic spores?

Sample weighing for MLT

What is the practice to weigh 10 g sample for MLT testing. Is it inside laminar air flow or it can be weighed somewhere in outside lab and taken inside thorough pass box then transferred in SCDM or BSCP inside laminar air flow.

Weight print is necessary or not?

A1: The sample should be weight inside the laminar flow hood, if there is any growth found in the test result; it will be difficult to judge that whether it is from the product itself or from sample weighing environment.

A2: To determine whether the environment had caused an OSS result for a microbial limits test, I would recommend that a passive Gravity Air Settling Plate be placed in the Laminar Flow Hood or Biological Safety Cabinet to ensure that laboratory error has not occurred during the testing of the sample from weighing it out, streaking selective agars from an enrichment broth and the performance of a plate count.

Entry exit procedures for MLT area

What gown should be worn in a class C area to test for MLT… sterile or non sterile clean aprons?

A1: Non-sterile gowns or lab coats are suitable for MLT.

Celsis false positives

For those of you that use a Celsis Advance II Rapid Microbial Detection system - what is your average rate of false positives (Celsis positive - no growth from enrichment tube)?

A1: If you have conducted a proper validation of the Celsius test method for a test sample in which you had determined whether the sample contains a high level non-microbial ATP that is at least twice the ATP level of the enrichment broth itself, you should not be obtaining a false positive test result for the presence of microorganisms.

If a sample contains a high level of non-microbial ATP by the inclusion of a natural raw ingredient such as starch, protein solution, natural gum, etc., you have 2 options. The first option is not to use the Celsius test method on those samples that contain a high level of non-microbial ATP for detecting the presence of microorganisms. The second option is to run an ATP bioassay on the sample that contains non-microbial ATP in enrichment broth and the cut-off for indicating a positive test result for the presence of microorganisms is twice the level of ATP of an unincubated sample that contains a high level of non-microbial ATP.

Finally, the length of incubation of a test sample in enrichment broth for an ATP assay is determined from your spiking studies of the USP indicator organisms listed in USP Chapters 60, 61, and 62 for demonstration of the recovery for all of the test organisms.

A2: Are you asking the rate if false positives from a Negative Control tube? Also, what threshold level are you using for a positive result?

Non-viable particulate excursions

I would like to know what industries do when having non-viable particle excursions in grade A areas.

A1: HAVC requalification is required .

A2: If the area is in operation, we would start by collecting a sample under static conditions. It that is also an excursion, then try to take some samples across the HEPA close to the filter face for investigation purposes to see if you can identify any issue.

Subculture on selective agar

I would like ask a pretty basic question about your approach regarding subculture on selective agar after selection with broth.

For example, in USP it's written for "Escherichia coli"

Selection and Subculture

Shake the container, transfer 1 mL of Soybean–Casein Digest Broth to 100 mL of MacConkey Broth, and incubate at 42° to 44° for 24 to 48 hours. Subculture on a plate of MacConkey Agar at 30° to 35° for 18 to 72 hours.

If there is not any color change in MCB, should we still subculture on MCA?

A1: Yes you would still transfer to MacConkey Agar regardless if you see a color change in Mac Broth. There are no other options in USP. The interpretation goes even further:

"Interpretation
Growth of colonies indicates the possible presence of E. coli. This is confirmed by identification tests. The product complies with the test if no colonies are present or if the identification tests are negative."

Note that it does NOT say brick red or even typical colonies - it says growth of colonies and you follow it up with identification tests.

A2: If there is no color change also we should subculture MCB onto MCA agar media.

USP doesn't say about observation interpretation of MCB media.

A3: Yes, absolutely still transfer. I have seen growth on MacConkey Agar even when it appears as if there is no growth or color change in the broth and then proceeded with identification.

A4: Are there specific identification tests or is always better to use ApiStaph or similar?

Using neutralizer for Purified Water tests

Do you use neutralizers when performing microbiological tests for PW?

If we could not obtain results without neutralizer during validation study for total microorganism and specific microorganism count, is it appropriate to interpret that water system is highly effective on not letting microorganisms live in the purified water system?

Or as in method suitability tests (e.g. DP/RM), should we try till getting recovery on plates?

A1: Purified microbial test method has not to be validated. Microorganisms obtained from culture collections are not adequate for this recovery. Use 1 ml Pour plate and R2A ir PCA.

A2: I am not sure why you would need to neutralize PW unless there is residual sanitizer in the system from a prior sanitization cycle, if this is being performed (e.g., residual Minncare, etc.).

Please advise how you treat your system.

A3: There is no need to use a neutralizer to test for the microbial content of purified water samples. There are no inhibitory substances in purified water unlike tap or potable water that may contain chlorine.

A4: As there is no added substances in PW to inhibit the recovery of bacteria no neutralizers would be necessary.

I would refer you to the AWWA/APHA Standard Methods for water and wastewater for details.

A5: If you have residuals of a disinfectant in a purified water system, I do not think that the water would still be considered as purified water. When I had to disinfect a purified water system with a disinfectant, I had to conduct rinsing of the purified water system and rinse the system until it was free of residues of the chemical sanitizer/disinfectant. This was confirmed that the system was free of chemical disinfectant residues by conducting conductivity and total organic carbon testing on samples taken from the system.

A6: What you have stated is common industry practice. However, the original poster complained they had issues with neutralization, hence my question about sanitization residuals not being removed.

Until the original poster responds, we will have no idea what the real issue is.

A7: Another concern for the "neutralization of purified water.”

Are you using ozone in your water system? If so, that may be causing your problem if it is not properly destructed. Perhaps there is a problem with your UV light.

A8: Referring to b/m response, I would like to know that we are using ozone in water system and there is UV light, so should we validated the method for recovery of microbes despite we are using membrane filtration method for water testing.

A9: Method of microbiological test for water has not to be validated.

A10: The sanitization process for a water system must be validated to demonstrate no additional substances are in the purified water.

With heat sanitization there would be no issues. Ozone treatment is transitory with ozone breaking down to oxygen which is accelerated by UV light.

Chemical agents are more problematic and must be flushed from the distribution system.

Present versus Positive

I would like to know if there is a standard way of reporting pathogens in a sample, either *present/absent* or *positive/negative*.

I've always used present/absent but would like to know if there is a rule, I'm having a hard time finding anything out there. I know FDA BAM suggests using present/absent.

A1: I think present/absent is the clearest way to report it, especially since <1111> lists the specified organisms acceptance criteria for each route of administration as "Absence of..."

On that same topic, I have had at least one FDA reviewer request we report results as "Absent per X" where X is 1 mL, 10 mL, 1 gram, or whatever so it's clear what amount of sample is being represented.

A2: I have always used Total Plate Count.

A3: I have seen it to be reported both ways for the USP indicator organisms that are pathogens. However, I prefer how it should be reported as in the FDA BAM as present/absent for each of the USP indicator organisms. The only problem in reporting this way is with potential objectionable microorganisms that are not listed in USP Chapters 60 and 62. With the exception of Ps. aeruginosa, Burkholderia cepacia complex and Staphylococcus aureus, the majority of product failures is due the presence of objectionable microorganisms that are not listed in the USP as a pathogen.

neutral pellets (sugar sphere) preparation

How do you prepare your sample (neutral pellets-sugar sphere) for TAMC/TYMC analysis? We perform a 1:10 dilution in buffered, 20 minutes in agitation. For the little size we have no problem but with bigger size we have a lot of spheres in the plates.

Are these sphere in plates a problem for inspection? I have no problem to distinguish between spheres or bacteria, but you know, sometimes it is hard to explain basic science during inspection without papers to support it.

A1: You can use dyes such as TTC to distinguish spheres and viable count.

A2: Adding the sugar spheres to warmed buffer or culture medium (not exceeding 45C) will add in dissolving the sugar spheres.

maximum filterable volume per a single sterility test canister

What is the maximum volume to be transferred per single sterility test canister, inclusive of pre-wetting, sample and rinsing cycle?

A1: The volume is more a function of the solution being filtered than the membrane. The canister can filter liters as long as there is no fouling/“plugging” of the membrane. If you wish to discuss in more detail, I would be glad to assist.

A2: I recall 250 ml bottles of 5% Albumin solution. Based on Tables 2 and 3 in USP <71> we would have tested 20 bottle with at least 10% of the volume for each media or 500 mL of product per canister. Filterability not volume would be the limiting factor.

A3: Your question isn't clear to me however i suggest the following :1.You can read USP chapter 71 (on Sterility test) 2. Number of rinse fluid should not exceed 500 ml irrespective of the quantity of sample tested. NB. If you have failures or an inconclusive result after the process (of 500 ml rinse), a validation of the process would be necessary (method suitability test).

USP 51 criteria

Can any body explain & interpret clearly with an example the following criteria from USP 51?

No Increase in counts is defined as NMT 0.5 log10 unit more than the value to which it is compared.

Positive aseptic simulation sample

If you have a positive purge sample during an aseptic simulation, can it be pulled from incubation on the day of transfer and not incubated the full 14 days? Also, if the vials are all negative, does the fill needle purge sample being positive effect the results of the media fill (camera footage shows that it was likely the sample cup).

A1: I think we need some clarification. If you have a positive purge sample, how would you know before day 7 or 14? Are you're testing a purge sample because you think it was contaminated by the sample cup or it's just part of your SOP? Either way, you still have a positive because the process wasn't aseptic. That being said, depending on the size of your media fill, there is some wiggle room where you can investigate 1 positive sample if you have more than 5000 units. An investigation is called for and you should "consider" another media fill. Above 10,000 units you can have one positive but will definitely need to investigate, however you don't have to invalidate the run. I have customers who don't follow this and will always redo the media fill if anything is ever positive, regardless of size.

In this case you're suggesting you have video proof of external contamination so that should help with the root cause. In my mind, you should clean and re-run the media fill because the process wasn't aseptic and that's the point of the entire exercise, even if you don't "technically" have any positive vials. Imagine yourself in front of an FDA inspector trying to justify your answer to get an idea if you feel comfortable with your decision.

A2: I have added some clarity...

For full accountability of the media, we collect the media used to prime the fill needles (to get rid of any air in the lines). This is not done for routine manufacturing. At a previous company, the MHRA gave us an observation for not collecting this material. The media was collected into translucent sample cups. We were able to see turbidity by day 7. We subcultured on Day 7 and returned it to incubation. Was it necessary to return it? Its not going to get more or less positive.

If this fill needle was truly contaminated, I would expect to see at least every 4th vial as positive (4 filling needles). We didn't see any during the day 7 transfer.

The footage showed an issue with the sampling with the likely source of the contamination (glue from the sampling cup tamper evident sticker). Do you think that coupled with this not being a routine production step is sufficient justification to not repeat (we filled about 5300 vials).

A3: Thank you for the clarification. No, I wouldn’t re-incubate because you want to get started on that investigation right away! You could always pull a sample and return it to the incubator if you wanted but at that point, a positive is a positive. In my single opinion, I would re-run the process out of an abundance of caution and to eliminate any questions from an auditor. That's only my opinion and I'm sure other people may have different opinions. I'd like to say "sure, invalidate that result"....but my gut tells me that's the wrong answer. At a minimum, your CAPA should correct the sampling issue.

Regarding resistance performance test

USP general chapter 55

I just wanted to know about the industry practice regarding d -value determination of BI.

Do you use BIs just by performing only enumeration or do you perform d-value determination of individual lots.

Also is there any guidance for testing BI at user end.

A1: We do enumeration and ID. We have the d values outsourced for a given lot of BI’s.

A2: What do you mean by outsource? You accept the values given by manufacturer or send a samples to third party for determining d value.

A3: Only spore enumeration is suggested for end user, when BI is used in accordance with the BI manufacturer's directions no D value determination is required as per USP 1229.

A4: We perform enumeration and identification on all incoming lots, per concentration, prior to use. If we use the tri-scale BIs, we do an enumeration on all three declared populations even though they are received as one lot.

USP 61 and USP 1227 for Pre-filtration Bioburden Methods

I am seeing some inconsistencies out there regarding references to USP 61 and USP 1227 for performing pre-filtration bioburden specifically for sterile pharmaceuticals such as eye care solutions. What I am seeing for USP 61 is the chapter is being used as a guide for the actual test method as well as qualifying the method. For qualifying the pre-filtration bioburden test as I have described above, shouldn't USP 1227 be used and referenced for qualifying the method? Method suitability would not be required in this case as per USP 61? USP 61 can be used as a guide but an SOP should be written for conducting the routine testing? I am interested in knowing what the industry practice is for qualifying methods and application of the above mentioned USP chapters for pre-filtration bioburden testing for sterile pharmaceuticals.

A1: Search "Microbial Monitoring For Biological Drug Substance Manufacturing: An Industry Perspective". This was consortium called BPOG that came up with a framework for testing pre-filtration bioburden. I've found it very useful.

Difference of Challenge organism in USP

I am just curious to know, What could be the reason for difference of typical challenge organism variation between USP monograph 1072 to 61and 62? And What if disinfection validation study used organism as per 61?

We observed difference for E. coli, Pseudomonas aeruginosa and S.aureus.

In-Process Bioburden Limits

I am wondering how companies are assigning their alert/action limits for pre-filtration bulk bioburden samples if data normally produces zeroes. Testing is performed as per USP <61>.

Is there an approach of considering the limits on the active raw materials/volume of WFI used to formulate/ validated filter SAL or is it simply based on trending alone?

A1: Search "Microbial Monitoring For Biological Drug Substance Manufacturing: An Industry Perspective". This BPOG working group talks about this very topic.

A2: The article was interesting, however I am still curious how companies are establishing their limits on parenteral in-process bulk samples.

A3: For Bioburden control, the general regulatory limit is: Less than 10 cfu/100 mL of solution.

Sterile compounds fix Alert limits according its historical data for TAMC and a specific limit for some organisms that have special size or characteristics that can pass through the filter.

A4: For the pre-sterile filtration bioburden specification, see the EMEA "Note for Guidance on Manufacture of the Finished Dosage Form" on page 5/6. It states that, for most situations, NMT 10 CFU/100 mL is acceptable. You should get a copy of this guidance for your records.

A5: [name redacted] has supplied a useful EMA guidance document.

A distinction must be made between bioburden control during downstream processing and bulk solution prior to sterile filtration.

The EMA NMT 10 cfu/100 mL limit is extremely conservative as a challenge to a sterilizing filter or control a bioburden that may add bacterial endotoxins to the product. Also it the limit for WFI.

A sterilizing filter validation requirement is > 10^7 CFU per sq. cm of filter area. Given the surface area of cartridge filter and the volume of the bulk product and bioburden would easily be 2-3 magnitudes higher, i.e. 10 or 100 cfu/mL.

In terms of downstream processing, most companies have a bioburden-reduction filtration after each major processing step and may maintain the product at 2-8 degree C to limit bacterial growth.

A6: A work around the 10 CFU/100 mL "prefiltration" bioburden is to insert a 0.45 um filter AFTER the compounding tank and take the bioburden sample after the filter. If starting with a relatively clean process a bioburden of 10 CFU/100 mL can be readily achieved.

Downstream, two, 0.22 um filters would need to be used as sterilizing filters. To summarize three filters are required: a 0.45 um filter (for bioburden reduction) and duplicate 0.22 um filters for sterilization.

I've personally used this setup and recommended it to clients; US, European and ROW agencies have accepted it.

A7: Pre-sterilising grade filtration bio-burden should be established based on historical data obtained from batches produced.

The guidance [names redacted] refer to has changed as of FEB 2018 and now read those limits have to be established using proper risk-assessment.

If your bio-burden prior to sterilising filtration is historically low based on data, I would suggest you put your alert limit on 2 cfu/ml and your action limit on 10 cfu/ml.

I agree with [name redacted] that whatever type of validated sterilising grade filter you use, it will remove any microbial contamination due to the fast amount of small pores compared to the number of micro-organisms the membrane is challenged with in real life.

A8: One word of caution on this approach - this will not eliminate the need to collect prefiltration bioburden from the first tank. You can raise the limit in the first tank and apply the lower limit only to the tank after the extra 0.45 um filter, but both tanks should be tested. US agency are now requiring this on review. The argument being that prefiltration should show worst case bioburden.

A9: Not sure I agree with your approach. If a manufacturer is having an approval in Europe as well, we have the PUPSIT requirement which is a pain in the neck for a redundant filtration set-up.

Likewise; in the approach you suggest you will have to sample for bio-burden prior to the bio-burden removal filter (0.45 um) as well as prior to the first sterilising grade filter to determine the actual efficiency of the bio-burden removal filter.

What a number of companies here in Europe do is they have two 0.22 um filter in series; the first one has a functionality for bio-burden reduction, the second one is the final sterilising filter. You still need to perform bio-burden analysis before both filters, but you have only one filter to perform PUPSIT (on the sterilising grade filter).

A10: I was referring to one compounding tank in which the prefilter is placed after (downstream) from it.

Personnel Monitoring

is this mandatory to perform the Personnel monitor the every person who enter in the core area during the batch filling (in Grade A and Grade B). Please revert with guideline reference.

Single Incubation Temperature for General Media Incubation

In the case of media fill vials, guidance calls for an incubation range of 20-35C for not less than 14 days and allows you to validate as appropriate with a single or dual temperature. I would like to know how companies are incubating their units and if dual, ensuring 20-25C incubation is performed first.

In the case of routine EM/PM sampling for aseptic process monitoring, there had been a shift in thinking over the past few years. There was a lot of discussion of changing incubation of general media at 30-35C for NLT 72 hrs followed by 20-25C for NLT 3-5 days to a single temperature (maybe 28-32) for NLT 72 hrs. Has anyone done this?

Has anyone ever had a citation from an auditor for incubating media fill units at the lower temp first but incubating the routine EM/PM samples at the higher temp first?

A1: I just finished compiling all of the FDA 483's for the last 5 years for a PDA book (available through the PDA website soon). In that five year period there are no citations for the incubation conditions you talk about. However, there have been comments/questions regarding the basis for the incubation conditions used, i.e., you should have data or published literature to identify why you chose the conditions you used.

Published literature gives several different approaches.

DuMoulan, et al. showed that a temperature of 32.5 deg C + 2.5 deg C was the best temperature overall for growth of microbes for sterility testing. (They were validating a rapid method)

Other publications say for biphasic temperatures to do the lower temperature first so you don't harm the fungi. Other compendia indicate that you should do the 30-35 deg c temperature first to protect the human borne organisms.

The best thing to do is to run an incubation conditions validation study. I published instructions for this type of testing in the IVT journal. None of my clients who have validated incubation have had any issues on incubation conditions from any regulator.

A2: Use one temperature that will not damage spores, yet will allow human bourne organisms to be detectable in a vial after 14 days. EM samples should be incubated similarly in my opinion. Qualify it.

Regarding USP 61

In our micro lab we are trying to validate a TVC method for a liquid per USP 61. USP 61 specifies under the recovery of microorganisms section for membrane filtration that the material filtered should represent 1 g of the product. However, under the "Amount Used for the Test" section it sates that "unless otherwise directed, use 10 g or 10 mL of the product to be examined." Would we be justified in only filtering 1 mL of product per the first statement?

A1: After reading the section of USP Chapter 61 called Recovery of Microorganisms in the Presence of of Product, it states that 1 gram or ml can be used for membrane filtration if you expect high numbers of microorganisms to be present. In sample preparation for water soluble and non-soluble products, it does states that a 1:10 dilution should be used. In the section, the amount of sample to be tested is 10 grams or ml's in which this sample amount is diluted in 90 ml's of diluent. If you are conducting membrane filtration of a test sample that contains a high number of microorganisms, I interpreted this read to say that a 1 gram or ml sample aliquot can be used instead. In most cases, I would expect that low levels of microorganisms would be present in a test sample. In addition, I would say that membrane filtration could be used on only those water soluble samples in which there were issues in neutralization of the preservative system by conducting a plate count method.

A2: I have a question regarding culture media pH, in USP (62) states that the pH of different media, if necessary, adjust before sterilization and after sterilization it is within specific medium pH range at 25 °C... My question here is, how we can measure the pH of agar medium at 25 °C whereas it is solidified below 45 °C... How we can use electrode to measure the pH of agar medium at 25 °C.

A3: You can purchase a flat-bottomed pH probe or you can sacrifice one bottle of media for pH measurement.

If you don't want to sacrifice the media, you can always aseptically pipette out an aliquot into a tube while the media is still liquid and use that instead after it solidifies.

USP 51

Can anyone explain & interpret clearly with an example the following criteria from USP 51?

No Increase in counts is defined as NMT 0.5 log10 unit more than the value to which it is compared.

A1: Convert the plate counts to log10 values. Take the difference between the two. It should not be more than 0.5 log10.

A2: If the difference of log between the obtained Count and the specified Count Is less than 0.5, the increase is considered method error.

pH of Agar Medium

I have a question regarding culture media pH, in USP (62) states that the pH of different media, if necessary, adjust before sterilization and after sterilization it is within specific medium pH range at 25 °C. My question here is, how we can measure the pH of agar medium at 25 °C whereas it is solidified below 45 °C. How we can use electrode to measure the pH of agar medium at 25 °C.

A1: They have flat probes for this. You can find them at any of your pH meter suppliers or large distribution companies.

A2: Get hold of a surface pH probe.

A3: You can use flat bottom electrode to measure Ph of agar medium.

Reference Docs for Biofilms in Water System

Are there any reference documents stating that heat treatment at 80C for 30 mins is effective against *all *biofilms in a water system?

A1: This is a good article addressing your question.

https://www.americanpharmaceuticalreview.com/Featured-Articles/345440-The-Problem-of-Biofilms-and-Pharmaceutical-Water-Systems/

A2: Running a loop alone at 80 deg C is NOT sufficient to prevent biofilms.

Some typical biofilm agents are strong chemicals or ozone Ozone can be routinely added to water and removed as part of the process. There is a lot of literature from ISPE and Nissan Cohan recommending this to prevent biofilms.

Strong chemicals work, but may be corrosive and create more nooks and cranny's that make nice homes for new biofilms.

A3: There is a nice paper on this in Biofouling (The Journal of Bioadhesion and Biofilm Research) by Montana State University team:

Wahlen, L., Parker, A., Walker, D., Pasmore M., and Surman, P. 2016 Predictive modeling for hot water inactivation of planktonic and biofilm-associated Sphingomonas parapaucimobilis to support hot water sanitization programs, Biofouling, 32 (7): 751-761.

A4: Biofilm will not be removed by heat treatment, better use 2% minncare chemical agent. Continued to 3days treatment will remove. Heat will reduce nos. And Not helpful.

2009 USP 51

Does anyone have a copy of USP 51 that was effective in 2009? If so, can you please scan and email to me at mjm@microbiologyconsultants.com?

A1: USP 29 (2005): http://www.uspbpep.com/usp29/v29240/usp29nf24s0_c51.html

USP 35 (2011): https://www.drugfuture.com/Pharmacopoeia/usp35/PDF/0052-0054%20%5B51%5D%20ANTIMICROBIAL%20EFFECTIVENESS%20TESTING.pdf

Not sure when 2009 version was replaced, but one of those should suffice, hopefully.

Bacillus cereus group contamination

I would be interested to know how others are handling Bacillus spore contaminations in your facilities? We have been experiencing a persistent contamination occurrence of certain Bacillus cereus group bacterium in several locations, but the origin / root cause is not yet clear. If others have had similar issues, I would appreciate your insight. What kind of sporicides did you use, e.g. Sodium hypochlorite (5000 ppm) or hydrogen peroxide (6 %). What about hydrogen peroxide vapour (6%)?

A1: From memory..

Hypochlorite should be 10,000 ppm to be fully effective, but will also be more corrosive.

Hydrogen peroxide at 6% will be very slow acting.

HPV is extremely effective if used correctly, the equipment needed is expensive.

Look at stabilised solutions of hypochlorous acid. Also two part chlorine dioxide products. These agents from reputable manufacturers are very effective.

A2: It will help if the source is identified and eliminated- but as for remedial disinfection, hypochlorite or a chlorine dioxide type sporicide (SporKlens?) is probably best- allowing sufficient contact time. Peroxide vapor is highly effective within a contained space, such as an isolator, but it is still a surface disinfectant and it's tough to get the right concentrations into every nook and cranny of a larger facility. Have you seen any increase in mold isolates during this same time period? This might help you zero in on sources-Good luck- Michael Carrollmcarroll7@juno.com Dear Forum,I would be interested to know how others are handling Bacillus spore contaminations in your facilities? We have been experiencing a persistent contamination occurence of certain Bacillus cereus group bacterium in several locations, but the origin / root cause is not yet clear. If others have had similar issues, I would appreciate your insight. What kind of sporicides did you use, e.g. Sodium hypochlorite (5000 ppm) or hydrogen peroxide (6 %). What about hydrogen peroxide vapour (6%)?

A3: Previously I said sodium hypochlorite at 10,000ppm. That's fast but probably too corrosive. 5000ppm should be adequate to kill spores with the correct wet contact time.

A4: I would highly recommend that you consider a hydrogen peroxide vapor decontamination service for a spore contamination. A vapor method (not fogging or spraying) can prove its effectiveness with the use of biological indicators versus relying on traditional EM methods that are very insensitive. Hydrogen peroxide vapor has a long history (since the early 1990s) in the pharmaceutical industry and is the gold standard for isolators. It is also used frequently in BSL-3 laboratories for equipment and HEPA filter decontamination.

How to establish statistical alert and action limit for water system performance?

Please suggest me how to establish statistical alert and action limit for water system performance? For the trend analysis I want to know how to establish the alert and action limit when the results are alway less than 1 cfu/ml. Please suggest what is the best way to calculate it.

A1: Refer to USP <1231> which suggests paying more attention to the trends in data for very clean water systems that usually have ZERO CFU as recovery. It's in the alerts and actions section (special) near the end of the chapter. A statistical approach may not be appropriate for you.

A2: Use non-parametric 95% and 99% Confidence Limits

A3: Use Cumulative Sum (CUSUM) charts which are most useful for identifying small shift in the process mean when an event—typically a change in average—took place. This is best for Micro environment monitoring to detct potential shift below 2 sigma.

1. A technique used to spot changes trend(s)
2. Good for large quantities of data with low counts
3. Helps visualize shifts in the process

A4: Actually, I've learned that using the 99% cut-off value approach to establish Action Levels can cause major headaches for very clean facilities. A couple of years ago I worked with a non-sterile facility that was squeaky clean. The resulting Action Levels were FAR below compendial limits. We decided to heed the guidance for Action Levels in PDA TR 13: “Typically the Action levels will be driven by the regulatory or industry guidelines. The Alert levels will be driven by historical analysis of the environmental monitoring data.”

You can use the 95% cut-off value approach to establish Alert Levels. Be sure you have a streamlined investigation program for investigating Alert Level excursions. Do NOT bombard your Manufacturing and Engineering folks with "hey, we got another Alert Level excursion!". That turns into white noise. The red flag should only be raised if you (the microbiology expert) find you an adverse trend (obviously it's always raised for Action Level excursions).

A5: Percentile works better... 95%

A6: We filter 10 ml from PW and calculate the result of: X average + 3 sigma, from the previous Year results, and declare, if the result is below 10 Cfu/10, than we apply 10 Cfu/10 ml as warning limit, and the double of it 20 Cfu=10 ml for action limit.

Longer period Incubation

Please guide me how to address Analyzed sample results (Water, Product, GPT) observation, sample enrichment periods/incubation timings has been increased by one day or two days due to sudden complete shut down. And let me know for this any guideline references are available.

A1: Compendia microbiological tests have defined incubation times and temperature so they are standardized and not open ended. Method suitability is usually conducted at the shortest incubation time.

If cultures are incubated at the specified temperature for long times, two major results will occur larger colonies on the plate which could result in the overgrowth of colonies or a large number of colonies. However, in most cases the plates will be usable and the results recorded.

A2: If you have exceeded pharmacopoeia recommended incubation then you can take following actions.

Retest all products where possible if it is not possible then my opinion as below.
1. For total aerobic microbial count and total yeast and mold count, see your plates if colonies are still countable and there is no swarming growth, no spreaders and no colonies merged then you can still use the results. For fungal plates it might not work.

While concluding your report it is better to write a justification for exceeded incubation period. Consider the trend of product and review of other related factors.

2. For specified microorganisms exceeded incubation time is not suggested as these microorganisms loose their characteristic features and possibly mislead the actual picture.
As far as guidelines are concerned they usually specify the incubation period.

A3: If there is a shut down during incubation you have altered the validated or compendial method requirements. In this case it is unknown whether this lapse in maintaining temperature will have an impact on the recovery of organisms, especially if they are stressed. You will either have to replicate the conditions and qualify the extended incubation or simply repeat the test with new samples (if possible and if this situation was a one-time event).

COVID-19 preparation of own hand sanitiser

What are peoples thought on using WHO details on preparing your own hand sanitiser? No microbiological incoming material bioburden or efficacy test data available.

https://www.who.int/gpsc/5may/Guide_to_Local_Production.pdf

is this ok to use for preparing locally for a manufacturing site to have its employees use in non GMP areas e.g. building entries, meeting rooms, toilets etc.?

Any immediate thoughts, concerns that should be assessed before it is implemented?

A1: Mitigating risk I recommend passing the sanitizer solution through a .45um or .22um filter to finish. Retain a portion of each lot for future testing. Volume to retain should be sufficient representation of the total lot/batch size. Retain small portion of starting materials too.

A2: Biocidal activity of ethanol and isopropyl alcohol are well established. They have lower efficacies against spore formers hence the addition of hydrogen peroxide the WHO formulation . They kill coronavirus within 30 seconds.

For example, the analysis of 22 studies on the persistence of human coronaviruses other than COVID-19 reveals they may persist on metal, glass or plastic for up to 9 days (Range 2 hours to 9 days) but they are readily inactivated by disinfectants and sporicides such as 62-71% ethanol, 0.5% hydrogen peroxide or 0.1% sodium hypochlorite within 1 minute (Kampf et al, 2020 J. Hosp. Infect. 104:246-251).

Other agents used in the pharmaceutical industry, including antiseptics 0.05-0.2% benzalkonium chloride and 0.02% chlorhexidine digluconate are less effective requiring a contact time of up to 10 minutes.

Given that we experiencing a COVID-19 pandemic companies could make their own hand sanitizer but if it used within a GMP facility I believe it should be manufacturers according to GMP requirements.

A3: FDA is allowing entities to produce hand sanitizers and does not intend to take actions against firms during this crisis. FDA has published two guidance industry documents:

The guidance "Temporary Policy for Preparation of Certain Alcohol-Based Hand Sanitizer Products During the Public Health Emergency (COVID-19)", is immediately in effect and outlines that the agency does not intend to take action against manufacturing firms that prepare alcohol-based hand sanitizers for consumer use and for use as health care personnel hand rubs during this ongoing public health emergency as described in the guidance.

The second guidance, "Policy for Temporary Compounding of Certain Alcohol-Based Hand Sanitizer Products During the Public Health Emergency", is in effect for the temporary compounding of certain alcohol-based hand sanitizers by pharmacists in state-licensed pharmacies or federal facilities and registered outsourcing facilities. Compounding is generally a practice in which a licensed pharmacist, a licensed physician, or, in the case of an outsourcing facility, a person under the supervision of a licensed pharmacist, combines, mixes, or alters ingredients of a drug to create a tailor-made medication. The temporary policy outlined by the agency does not require compounders to obtain a patient-specific prescription.

A4: I suppose it depends on your situation if you cannot obtain alcohol hand sanitiser.

But if you have to manufacture in house I am sure you will need efficacy data for regulators. Also stability and shelf life etc.

Some points to bare in mind when manufacturing..

Alcohol is not sporicidal you may want to sterile filter the solution prior to use. And ensure it is spore free.

Yes this formula contains peroxide but at that final concentration that would take several days to act on any spore contamination in your finished product. Dont rely on the peroxide to do the job of filtration.

I do worry about operators using product containing peroxide on the hands over a prolonged period even at this low level. You may want to leave out the peroxide and rely on filtration and testing to ensure spore and other microbial contamination free.

Use volume not weight during manufacture as alcohol in particular has a different SG to water. It complicates things.

If you are not used to manufacturing alcohol products on site your facility will have to be designed to handle flammable products.

A5: The History Of 0.125% Hydrogen Peroxide

Just a bit of additional background info. Why WHO add peroxide.

20 or so years ago in UK, but probably not US, there was a 70% ethanol product sold widely to hospital aseptic units. It was cheaper than irradiated alcohol and slightly safer than non irradiated alcohol with no additions.

It was 70% ethanol plus 0.125% hydrogen peroxide. WHO seem to be adopting this strategy with their hand sanitiser.

It is possible for unfiltered alcohols to contain spores. The point was that unfiltered alcohol could be dosed with 0.125% h2o2, packaged and if left for 7-10 days any spores in the preparation are destroyed. So the alcohol could be deemed spore free and could be tested to show than.

Packaging was not sterile, content could not be claimed to be sterile. But it was widely used.

A surprising down side was the preparation did attack some grades of stainless steel manifested as rust. I expect the alcohol evaporates, leaves the peroxide at a higher concentration. Over time this causes corrosion.

Also some operators complained of sensitivity to the product.

Eventually regulators started to discourage its use especially in A/B areas and it fell out of favour and is no longer used. It wad never used in non Gnp areas of course.

I dont think it will add significant biocidal properties to the product. Especially with such a short contact time. There are many hand gel formulations that don't use peroxide.

A6: I have been following this conversation and find it to be very interesting about the preparation of an alcohol hand sanitizer by using the WHO guideline. In looking at the composition of this Who alcohol hand sanitizer, the most likely source of Gram-positive bacterial contamination would be from the glycerol that is used in the formation as an ingredient at a 1.45% concentration and un-filtered alcohol. It is also highly unlikely that a concentration of 0.125% hydrogen peroxide in the formulation will not keep the formulation adequately preserved but is used mostly to take care of spore-forming bacteria that are present in the formulation at time of compounding. In general, hydrogen peroxide will break down in a product formulation if it is not stored in a proper package that prevents light exposure and if not stored at a proper temperature. Hydrogen peroxide is not found to be used as a common ingredient in most commercial alcohol hand sanitizers. Glycerol is used as a moisturizing agent in alcohol hand sanitizers due to the drying action of the alcohol on the skin. However, most people would consider an alcohol hand sanitizer product formulation to be self-preserving present against non-spore forming microorganisms that could be introduced during usage of the formulation with a concentration of 80% Ethanol or 75% Isopropyl alcohol

It should be noted that alcohol hand sanitizers with a concentration of alcohol between 60 and 95% are considered in the United States as Over-the Counter (OTC) drug products. If you look at the recent article in the Journal of Hospital Infection (Kamf, G., Todd, D., Pfaender, S. and Steinmann, E. Persistence of Coronaviruses on Inanimate Surfaces and their Inactivation with biocidal agents. J. of Hospital Infection 104 (2020), 246-251), most coronaviruses are inactivated within 30 seconds of exposure in a conducted suspension test. It seems that the recent document from FDA had been issued due to issues in obtaining alcohol hand sanitizers. If you read the FDA compounding document, it states that you will need to wash your hands for at least 20-seconds with soap and water. If soap and water is not available, I do not think that using an alcohol hand sanitizer for 20-seconds might be enough to inactivate coronavirus based upon the information in the J of Hospital Infection article.

It should also be noted that FDA will not allow manufacturers to make viricidal claims for OTC alcohol hand sanitizer product formulations. However, I believe most people are using alcohol hand sanitizers for this purpose due to this pandemic by SARS-Covid-2 virus in addition to soap and water.

Vendor QC criteria for selective agars

We have struggled with recovering Pseudomonas within the minimum 18 hours per USP <62> when performing growth promotion for Cetrimide agar. We recently have updated our procedures to set a minimum time of 24 hours for all Pa testing which has helped some, but we've still failed to recover Pa even after 24 hours of incubation. We recently filed a complaint with a media manufacturer for a couple specific lots of media we could not get to pass. During the investigation I learned that their QC procedures have an 18 hour read for growth promotion, but if there is no growth then the plates can be re-incubated until growth is observed.

I'm curious what others' thoughts are on this practice, and if you would view this as an acceptable practice by a media manufacturer. If the media is made per the USP chapter formulation but the manufacturer fails to recover the target organism within the minimum time specified in the chapter, how is that acceptable?

A1: When I was there, we saw the same type of issue with Cetrimide agar. I think the incubator that is being used is on the lower side of the required temperature for this media. You may want to look at that. If you are referring to Remel Cetrimide, we use it at Winchester and don't have issues with recovery. Our incubator stays about 33C. Something to consider.

A2: USP <62> states that for growth promotion that the plates should be incubated at the specified temperature for the shortest period of time specified in the test. For P. aeruginosa on Cetrimide agar with would be 18 hours at 30-35 degree C.

When I consult the 2003 Disco/BD Manual I find that the culture response for Difco Cetrimide Agar base is P. aeruginosa ATCC 27853 with an inoculum of 10^3 CFU incubated at 33-37 degree C for 18 to 48 hours is a good recovery of yellow-green to blue colonies. This is a claim that the medium meets USP specifications.

Variables that may differ with the <62> instructions are the ATCC culture specified, inoculum size and presentation, the incubation temperature and the incubation time.

A3: If you are doing growth promotion on selective agar by inoculating with < 100 CFU you may well get failure to recover. This is not how the selective agar is used in USP <62> testing. Rather you inoculate with much higher microbial content after enrichment and you are not concerned with colony count but recovery of the organism (absence is the criteria). Recommendation is to perform enrichment as in the USP <62> test, then inoculate your cetrimide agar. If you get growth of the Pseudomonas aeruginosa then your agar is acceptable for use.

A4: Carry out an OOS investigation, questions like this /steps can be helpful :

1. Is the organism viable?
2. What passage did you use?
3. For P. aeruginosa, incubation at 30-35 degrees is ok, but you can also incubate at 42 degrees.
4. Was the media prepared properly?
5. Are you doing a quantitative or qualitative test? As a selective medium it should be qualitative, hence you can streak the organism directly on the agar and incubate.

A5: Selective media often is unsuitable for enumeration. When the USP wrote (2022) growth promotion was determined by streaking the inoculum on to the plate not using 100 CFU.

A6: In my experience this is a concern shared by so many non-sterile facilities.

Growth promotion testing (GPT) of selective media following Microbiological Examination of Non- Sterile Products: Tests for Specified Microorganisms Ph.Eur.2.6.13 / USP<62> / JP 4.05 can be quite challenging.

Selective media are designed to provide a conducive environment for growth of some types of microorganisms but not to others. They provide the ability to control what will and will not grow on a culture plate. However, it is important to note that a microorganism will not grow as well on a selective media compared to a non-selective media. This is entirely due to non-selective media being well balanced with all the elements that most bacteria need to grow and flourish, whereas in selective media, due to the presence of either activating or inhibiting elements, selected microorganism will/can grow but not thrive.

Therefore, compendial test requirements for selective media are not quantitatively evaluated. Instead, pharmacopeia requirements for selective media testing state: "Growth of the microorganism comparable to that previously obtained with a previously tested and approved batch of medium occurs." No percent recovery requirement must be achieved when testing a selective media batch. The qualification is based on growth of the new batch of selective media comparable to growth of previously tested and approved batch of the same selective media. But also be aware that selective media can always vary batch to batch even in the best manufactured situation.

A7: USP <62> states: 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〉 <https://online.uspnf.com/uspnf/current-document/GUID-0392F79D-1F8A-4B8D-BEC8-C6FD7B39966F_1_en-US>), 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.

This is a qualitative acceptance criterion.

In contrast USP <61> states: 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.

This is a quantitive acceptance criterion.

A8: Both you and [name redacted] mentioned inoculating with a higher level of organism, however USP <62> states the following under Test For Growth-Promoting Properties, Solid Media:

"Perform Surface-Spread Method inoculating each plate with a small number (not more than 100 cfu) of the appropriate microorganism."

Would an auditor not question why we are deviation from a NMT 100 CFU inoculum if we used a higher level for growth promotion of selective agars?

I understand that selective agars are not meant to be used quantitatively and that during routine testing you're streaking from a pre-enrichment, but USP is pretty specific in this section.

A9: Here is a great link that talks about growth on non-selective media:

https://blog.microbiologics.com/9-tips-for-growth-promotion-testing-on-selective-media/

ENC: Endotoxin recovery from cleaning validation

I count on your help, I need articles and reference on how to validate a method of recovery of bacterial endotoxin focused on the study of Cleaning Validation in the pharmaceutical industry.

A1: To start, you should review USP Chapter 1228.

A2: I hope this helps, doe i must say your question isn't clear? Are you asking for method of validating a cleaning method for pharmaceuticals? and validation of endotoxin test methods?

I have not read about recovery of endotoxin in cleaning before, unless you are talking about recovering of gram negative organisms (are responsible for realize of endotoxins) then it becomes clear to me. However i would like to be enlightened if you have more information on your questions that i haven't answered.

A3: This may be a reasonable approach.

With a cleaning validation protocol we determine worse case products in terms of a challenge to the cleaning process and risk of cross contamination. In the later you would consider solubility, toxicity and low dose-high potency drug products.

Your chemical analytical method would be validated to recovery the active ingredient with a limit of detection commiserate with the risk. The methods employed are usually a swab method but in some cases a rinse method.

For endotoxin you would need to establish an endotoxin limit in terms of EU per unit area of the processing equipment. You would show that in sample obtained in the chemical assay does not interfere with the recovery of an endotoxin spike.

A4: The question is the development of a specific method for quantifying endotoxins by rinsing in the construction materials of the collection points?

A5: I assume you are referring to endotoxin recovery from surfaces (and not recovery just as part of the analytical method itself). If that is the case, endotoxin recovery from surfaces (like recoveries done for actives in pharma cleaning validation) is not typically done, nor is it appropriate (except perhaps as a research study). Endotoxin recovery from surfaces is highly variable, and even if you got percentages, what would you do with them? Typically, for cleaning validation purposes people set limits for endotoxin in the final rinse water, and use the WFI specification as the limit.

A6: What volume of rinse water with respect to the processing equipment?

A7: I've never read something about this kind of recovery study for cleaning validation.
My opinion is : you have to apply the pharmacopeia specifications on the last rinse water used in your cleaning process.
So if you rinse with WFI, your acceptance criterion in cleaning validation will be <0.25UI/ml on your final rinse water.

A8: 1. Is there any study on effectiveness of sanitation tunnel to be used for pharmaceutical employees ?
2. What are the disinfectant can used ?
3. What will be concentrations of disinfectant and contact time ?

A9: The volume of the rinse water sampled should not exceed the working volume of the equipment.

Query related to Environment monitoring acceptance criteria for settle plate and microbial air sampling of LAF/BSC located in Grade C,D and unclassified area.

I have query related to Environment monitoring of LAF/BSC located in GradeC,D and unclassified area,

How can we defined acceptance criteria of LAF/BSC located in Grade C,D and unclassified area for settle plate and microbial air sampling.

A1: A LAF/BSC would be expected to meet Grade A requirements.

A2: If your lab is GMP approved and if it is DMF filled you cannot degrade or change the classification of the clean room area but if your product is not DMF filled you can degrade the clean room classification by considering risk and potential safety to the product.

A3: Refer WHO guideline for environmental monitoring to clear the doubt.

NVP Spike

If we wanted to spike the air to reach a 0.5 micron non-viable particulate level of 35,200,000, what would be the best way to achieve that?

A1: When we perform ISO testing and verify room recovery rates for our customers, we use a fogger to smoke the room to get the high particulate counts and then monitor 5 and 0.5 micron counts with Climets. We try to use a water based fogger (vs. glycol fogger) for clean-up purposes.

A2: The idea is to try to determine how quickly an area can recover from a sudden increase in particles.

A3: Using fog to generate particles is not reflective of real conditions in a cleanroom. Particles in an ISO 5 controlled facility do not appear as an uniform size and distribution phenomenon.

Cupriavidus pauculus

In a sub-loop of one of our Purified Water systems, we have found Cupriavidus pauculus to be the culprit of a contamination.

We have not seen this bacterium before in our plant; hence the question to the forum: is anyone familiar with this bacterium and how does it relate to bio-film formation in PW distribution systems?

A1: The taxonomic name change - Cupriavidus pauculus was formerly classified as CDC group IV c-2, Ralstonia paucula and then as Wautersia paucula.

It is a waterborne Gram-negative bacterium very rarely associated with infection

A2: A check of the literature indicates that this genus was at one time Burkholderia

Cupriavidus pauculus,

Cupriavidus pauculus is a Gram-negative, nonfermentative, motile bacterium of the genus Cupriavidus and family Burkholderiaceae isolated from water from ultrafiltration systems and bottled mineral water. C. pauculus is associated with human infections.

Wikipedia notes
Scientific name: Cupriavidus pauculus
Genus: Cupriavidus
Family: Burkholderiaceae (previously Pseudomonas)
Class: Betaproteobacteria
Order: Burkholderiales
Phylum: Proteobacteria

This genus of bacterium has long been associated with colonization of purified water systems. There are various items that need to be troubleshooted like distribution system design, water temperatures , sample ports, water production systems (RO versus distillation), etc.. that are part of the investigative triage that microbiologists go through to solve issues like this. Start with some research in the areas of the previous naming of the microorganism and you may find much more information of how to diagnose and remediate the water system you are investigating.

Sterility Testing of Gowning Apparel

How is industry performing USP chapter <71> sterility testing (including method suitability) to confirm a vendor's claim of sterility on incoming gowning apparel?
Ex. Gloves, coveralls, boots, face shields, etc.

A1: Why? Are you not performing personnel monitoring on your operators?

A2: For a start USP <71> is directed to pharmacopeial articles not gowns.

The sterilization of gowns would be validated and each lot subjected to parametric release.

Furthermore if the gowns were not sterile you may expect to see higher counts during personnel monitoring.

A3: Please don't get me wrong - while I completely understand that <71> is certainly not intended for this type of item, some non-microbiology persons cannot understand/accept the difference between the word "sterile" and the requirement for "sterility testing".

Nonetheless, my last resort - after wasting a lot of time and money on this initiative - is to see if anyone else in the world is performing this, and if so, how.

A4: The only time I have ever seen sterility testing of gowning materials (gowns, hoods, gloves, etc.) was as part of sterilization process validation. Never by the end user.

A5: True, not tested by end user, However, need to ensure that supplier should provide gamma radiation certification and validation whenever end user requested, in addition, it is better to qualify supplier as approved for less hassle.

A6: I do not align with all the replies indicating this is nonsense. i too have had FDA inspectors ask for sterility testing of gowns and gloves etc as part of supplier qualification. I was also at a company recently who had training being delivered by a USA industry expert who trains the FDA inspectors, and she talked of this very thing and how to verify gowns are sterile from the supplier. USP <71> is the method used to demonstrate the items are received sterile. The process is to cut a sample from the gown (with sterilised scissors) and test as per <71>.

This is not a routine test, but is expected (by some regulators) as part of your supplier qualification.

A7: Sometimes the FDA investigators are misguided and encourage bad science.

A8: If you are cut a sample of gown and performing a sterility as per USP<71> then what about sterile gloves?. Because previously we used to perform sterility of gloves by cutting the sample but another USFDA inspector suggested to perform direct inoculation method. So which one is the correct?

A9: True. i have seen both methods employed. The problem with direct inoculation (as requested by inspectors) is that they trap air and float. So not all surfaces are covered. Then you get another observation for not completely submerging them.

A10: No. The gloves whichever we are inoculating as per direct inoculation, it never floats and it will be submerged below the aerobic environment. The issue is which method should be followed?. Is there any guideline says the methodology?.

Biological indicators for Low temperature 115

Which is the best biological indicators for heat sensitive products as per European regulatory agencies? In USP it is clearly mentioned to use Bacillus subtilis.

Growth promotion for TSB

Does anyone do growth promotion of TSB test tubes used for BIs with *B. atrophaeus *and *G. stearo* using a low level inoculum? It seems to be the right thing to do but we are struggling finding an outside lab that does it or the purchase of low level inoculums such as Quanticults to do it in-house (we do not have the capability of making our own stocks just yet).

A1: No need to perform growth promotion test with the organisms you mentioned. Growth promotion of TSB tubes to be done as described in USP general chapter 62.

Also most of the media manufacturers provides COA with organisms you mentioned.

I would suggest you perform the audit of manufacturers/ vendor approval. Then consider growth promotion test done as per manufacturers COA.

A2: Typically there is a positive and negative control used for each run of Bis. The positive control is simply an unexposed BI in the media tube-this demonstrates that the media was sufficient in promoting growth. Hope this helps-

A3: The issue is whether the growth promotion test requirements outlined in USP <61> or <71> would be adequate for the recovery BIs incubate at 60 degree C.

Typically the QC microorganisms represent a wide range of microorganisms that could be isolated and a QC test for efficacy of each incoming lot of the medium.

In labs I have managed we have run a positive control of unexposed BIs.

I believe this is adequate.

A4: Yes. You can get suspensions of each and inoculate < 100 spores in the tubes. Especially useful if you have concerns the BI may harbor residuals, such as VHP. In this case, expose an uninoculated BI carrier, place in tube and add the spore suspension.

A5: [name redacted] has a good point. With dry heat or moist stream there is no residues but with ethylene oxide or vapor phase hydrogen peroxide there may be. With irradiation dosimeters not BIs are employed.

With sterilized product your have aeration time or test for EO residuals with material with a high capacity for EO adsorption.

The effect of VPH on the BI matrix could be investigated prior to process validation.

However, I do question the use of BIs in validated, routine sterilization runs.

Neorickettsia risticii?

Does anyone know where I can purchase Neorickettsia risticii for a liquid disinfection study? ATCC does not have it.

enumeration of non-lactic

Anybody can suggest the media& buffer suitable for enumeration of Non-Lactic bacteria? (ISO 13559 IDF 153)

Cell Therapy Risk Assessment Article Published

Risk Assessment Approach to Microbiological Controls of Cell Therapies <https://journal.pda.org/content/74/2/229> Tony Cundell, Scott Drummond, Irving Ford, Dona Reber and Donald Singer; members of the Pharmaceutical Microbiology Expert Discussion Group PDA Journal of Pharmaceutical Science and Technology March 2020, 74 (2) 229-248; DOI: https://doi.org/10.5731/pdajpst.2019.010546

A1: Excellent paper! I have been asked how best to assure that cell therapy products are safe and have used many of the criteria that are in this paper to provide guidance. Now I have a definitive paper to cite. Great job!

A2: Can anyone had experience of analysis of colour injection for sub visible particulate matter test,. Is there any guideline which speak about colour injection analysis for sub visible particulate matter.

Testing of Disinfectants as per BS EN 1500;1997

If anyone has performed testing of Disinfectants as per BS EN 1500, i need some help.

A1: This method is similar to ASTM E2755 , What help you need?

A2: You will struggle at the moment because you require candidates and with social distancing things very difficult.

You may have to select EN1276 (using hand hygiene strains) with EN1500 at a later date.

A3: In my opinion, during a global pandemic, the pharmaceutical industry should be able to make a like-to-like disinfectant substitution if it necessary to make a supplier change due to shortages without conducting disinfectant validation studies.

Rapid testing method for sterility testing

I need your support to know which is the best or FDA approved rapid testing method for the sterility test of sterile products, an alternative to USP <71>.

A1: The FDA does not "approve" alternative methods. I would recommend that you reference USP 1071.

A2: There are multiple systems that have been approved for use by the FDA. Each system has it's own advantages and disadvantages. The FDA doesn't particularly approve each system, rather it approves the alternative method for a specific product based on validation submitted by the manufacturer.

Dr. Miller's website has information on all these methods: http://rapidmicromethods.com/

A3: FDA will approve validation protocols submitted by the end-user, but does not normally approve the methods themselves, unless utilized as diagnostic devices.

For pharma rapid sterility testing, the choice of the system will depend on a number of factors, including the sample matrix, the desired time to result, level of sensitivity and other factors.

To help you decide what might work for your product, I would advise you to visit the tutorials on technologies and rapid sterility testing at http://rapidmicromethods.com. There is good section on ATMPs and the recent regulatory guidance for rapid sterility testing of these types of products.

USP <60> Question

USP Chapter <60> on testing for B. cepacia references three (3) different strains of Burkholderia for Growth promotion and Suitability testing. Are your labs using all 3 of these strains each time you perform these tests (GP & Suitability) or do you select only one , such as the hardest to recover?

A1: Right now all three strains. No plans to use one strain.

A2: We use all three for growth promotion of the media and method suitability.

A3: The 5 organisms in the chart should be used for GP and Suitability testing. I believe the "or" is a mistake in the chapter and perhaps Tony Cundell can confirm that.

A4: Do you also perform this testing routinely on your water systems?

A5: It is a requirement for USP Chapter 60 to use all 3 indicates Burkholderia strains for growth promotion and suitability testing. Burkholderia cenocepacia and Burkholderia multivorans are slow growing strains that needs at least 48 to 72 hours of incubation to be detected when conducting suitability testing.

A6: I can understand as to why you would use the indicated 5 organisms for growth promotion testing of BCSA. However, Pseudomonas aeruginosa and Staphylococcus aureus will not be inhibited for growth by using Soybean-Casein Digest Medium with Polysorbate 80 and Lecithin during suitability testing of Chapter 60. I would use the 3 Burkholderia strains to perform suitability testing of a non-sterile aqueous product formulation if conducting USP Chapter 60 testing. I can see using Ps aeruginosa and S aureus for suitability testing of the product has the absence of these 2 organisms in addition to the Burkholderia strains.

A7: The USP chapter is entitled <60> Microbiological Examination of Non-sterile Products: Test for Burkholderia cepacia complex.

The test was not designed for purified water monitoring. Bcc can be isolated on R2A agar.

A8: Very good question. In general, isolates from purified water systems are stressed due the presence of low nutrient conditions that are present in these systems. I do question as to whether BCSA could be used to recover isolates from purified water systems because BCSA is a nutrient rich medium in comparison to R2A Agar and Plate Count Agar, low nutrient mediums, that are used to recover isolates from purified water systems. It could be that BCSA could inhibit the isolation of purified water system isolates due to its nutrient content. In addition, I wonder whether the selective agents that are used in BCSA to isolate Burkholderia cepacia Complex isolates could also be harmful to recover nutritionally stressed isolates of this complex from purified water.

A9: We use all three organisms for both GP and suitability testing.

A10: What's the specification of Bcc in product/ water sample? Should be absent in 10g (or) 10ml of sample?

A11: I specifically asked this question at the end of the PDA Microbiology show with the FDA panel and it was very clear there is no expectation for firms to be testing their water systems on a routine basis. This is meant for aqueous, non-sterile products only.

A12: The chapter states "NLT 1g"...not less than one gram of product.

A13: Do you think the TSB enrichment would hurt or help the Bcc recovery from water? The APR study that was done by Sutton, Barlasov, and Jakober showed that enrichment worked for Bcc with TSB and plated on a variety of media.

A14: Relating to this, are companies performing retrospective validations on products now that testing the 3 strains is a requirement for suitability? Or is just for new products moving forward?

A15: I recommend reading:

Glowicz et. al. American Journal of Infection Control. June 2018. 46(6) 649-655. A multistate investigation of healthcare-associated Burkholderia cepacia complex infections related to liquid docusate sodium contamination, January - October 2016.

Bcc was isolated from drug product and water samples using a TSB enrichment and BCSA plating method that was very similar to those recommended in USP <60>.

A16: In reference to your question as to whether TSB could be used as an enrichment to isolate Burkholderia cepacia complex from purified water samples, I aware of the article in the American Pharmaceutical Review called “Recovery of Stressed (Acclimated) Burkholderia cepacia Complex

Organisms” by Barlasov, Sutton and Jakober that had been published in 2014. In the article, they did stress the Burkholderia cepacia complex test organisms by storing them at 2 to 8C in Sterile USP Water at a concentration between 1000 to 10,000 CFU/ml in which a 1:10 dilution was made and there was the detection of sufficient growth on different types of agars by using TSB with Lecithin and Tween as an enrichment broth by using a 48-hour incubation period at 30 to 35C. Enrichment in TSB could be used, but an enrichment step is generally not performed when conducting the microbial analysis of purified water samples. If a selective/differential agar is used, a 100-ml aliquot of a purified water sample is filtered by using either a 0.22 or 0.45-micro filter, the filter is rinsed with sterile deionized water and the filter is placed onto the agar plate. Without an enrichment step, I do have questions as to whether BCSA could be used to detect nutritionally stressed Burkholderia cepacia Complex organisms directly from a purified water sample. It is not unusual practice to use an enrichment step to recover stressed organisms from a test sample.

In reference to one of your postings that you had indicated that an FDA person said that they would not expect to use USP Chapter 60 to test for the presence of Burkholderia cepacia Complex in purified water samples, it will not prevent an inspector from saying so that a company must use the method. Unless it is explicitly stated in the USP Chapter 60 that this method in USP Chapter 60 s should not be used to test purified water samples which it is not, it will not prevent an inspector stating that this method should be used. We have seen many times in which an inspector had recommended the use of an inappropriate method for a test sample.

A17: Of course normally we would test 100-200 mLs of purified water directly on R2A, etc. USP 60 says "for example, a 1:10 dilution may be required when conducting optional testing of pharmaceutical waters." It doesn't make sense to me to perform a dilution on pharmaceutical water that will go onto a selective media. Even if the total microbial flora is high (which it shouldn't be), much of it (gram -) would still be inhibited by the BCSA.

We have [name redacted] on the list….[name redacted] can you confirm the enrichment/non-enrichment of "optional pharmaceutical waters"?

Also, I specifically asked the FDA panel about testing water because I had a feeling this would come up and be confusing. I am most definitely not trying to create another "testing for thermophiles" situation!!

A18: My take on the interpretation of the "a 1:10 dilution may be required when conducting optional testing of pharmaceutical waters" text in USP 60 is a dilution of the TSB enrichment, and not the water itself.

For example, filter a water sample then submerge the filter in a suitable volume of 1:10 dilution of TSB. Then subculture onto BCSA after enrichment.

Since water microbes struggle to grow in high nutrient media, diluted TSB can be more successful recovering them. We've been doing this for a while to revive bioburden recovered on R2A to prepare the cultures for identification. In the lab we jokingly refer to the medium as "Diet TSB". :)

A18: In thinking about the use of USP Chapter 60 for testing the presence of Burkholderia cepacia complex in pharmaceutical waters, I believe that the use of a TSB enrichment of a water sample is not present in “Standard Methods for Water and Wastewater Analysis” by APHA. In general, the microbial test methods in this book are considered to be validated. With a TSB enrichment method not listed for conducting the microbial analysis of water samples in this reference book, I suspect that you would have to conduct suitability testing on your water samples. Do you believe that this is a correct interpretation by my thinking?

The question about what a 1:10 dilution for the analysis of a water samples is a good one. I would think that it would mean that a 10-ml sample aliquot is added to 90-ml of TSB, but this is just my perspective.

A19: I contacted USP for their take on the interpretation of the 1:10 text and already received a response. In short: "Diet TSB" is the intent according to the USP liaison for USP <60>.

Here is the full exchange:

"Hello again [name redacted],
I hope you are doing well in these unusual times.
My question today is again regarding USP <60> text. There is an active discussion on PMFlist regarding some text found in USP <60>. Can you please clarify the text describing the use of a 1:10 dilution when testing pharmaceutical waters?

Interpretation #1: The 1:10 dilution refers to a very dilute TSB enrichment to which sample material is added (for example, add the sample to an enrichment that is 10 parts TSB and 90 parts sterile water).
Interpretation #2: 10 mL of water is added to 90 mL full strength TSB for resulting in a 1:10 dilution of the sample.

There is a vast difference between the enrichment in the two interpretations. The first one being a very dilute TSB enrichment with sample added. The second one uses standard full strength TSB with sample added.
As always, thank you for your input."

[name redacted] response:
" Dear [name redacted]-
Thanks and wish the same of you.
Option#1."

A20: if you read USP <60> I believe you will determine that the 1:10 dilution applies to the medium and not the water.

A21: Thanks for the clarification [name redacted]. I hope this gets out to as many non-sterile aqueous manufacturers as possible so there is no further confusion.

A22: 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?

A23: We are having a similar experience with the appearance of these organisms on BCSA. The statement in USP <60> regarding the presence of white colonies surrounded by a pink-red zone on BCSA is under the Interpretation heading in the Testing of Products section. Perhaps there are other members of the Bcc that produce white colonies on BCSA, but we are not seeing that with any of the three USP <60> ATCC test organisms.

The growth promotion section of USP <60> states, "Colonies are comparable in appearance and indication reactions to those previously obtained with a previously tested and approved batch of medium." Our approach was to observe the appearance of each test organism on BCSA and use those observations as the basis for normal appearance and indication reactions for growth promotion testing of all future lots of BCSA received.

A24: In USP Chapter 60, it states that the 3 Burkholderia strains should be growth promoting and indicative on BCSA. However, there is nothing in this chapter that states what should be the indicative reaction for each of these strains on BCSA.

If you look at the product informational sheets for BCSA from different suppliers, they only use Burkholderia cepacia ATCC 25416 as their growth promoting test organism. I have not seen any media

manufacturer data indicating what would be the appearance of Burkholderia cenocepacia and Burkholderia multivorans on BCSA. However, I have also seen media manufacturer literature that says that Burkholderia cepacia complex colonies will appear as either white colonies surrounded by a pink-reddish zone or greenish-brown colonies with a yellow zone on BCSA. Because it is very difficult to separate the members of Burkholderia cepacia complex by biochemical means, you may need to have use this either/or appearance of colonies on BCSA. For this reason, I believe that it was assumed by the USP that the growth of Burkholderia cenocepacia and Burkholderia multivorans on BCSA will appear the same as Burkholderia cepacia ATCC 25416.

It is best to use the guidance in the USP Chapter to base the indicative reactions for each of the 3 strains of Burkholderia in how they appeared on a previous batch of BCSA as your indicative properties.

This problem in the appearance of Burkholderia cenocepacia and Burkholderia multivorans on BCSA is a good reason for not using colony appearance on a selective/differential agars as a presumptive identification of an isolate. When it comes to identification of microbial isolates, it is time to move into the 21st century to use the technology that is available today and do away with presumptive identifications of isolates. It would be great if the Star-Trek Tricoder would be available to do identifications of microbial isolates.

A25: It's also worth nothing that <60> states: "Any growth on BCSA is confirmed by identification tests".

Because the differential properties are not very good, we simply record any colonies as "growth", and then report absence/presences once isolates have been ID'd.

A26: Can you tell me if you know whether most companies are testing their water on a routine basis even though it is not a requirement? Are they using absence of BCC as the requirement for their purified water?

I am also interested in whether companies are testing all of their liquid products for BCC as a standard across the board or are they using risk assessments of their systems to determine whether this testing is required for each of their liquid products. If companies did not initiate product testing when the USP 60 became effective, are they going back to test product from the date of effectivity?

A27: I can't comment on what most companies are doing with regards to BCC because the guidance just came out 6 months ago. I do know that prior to that USP chapter many (most) non-sterile MFG were monitoring their water for gram (-) bugs and some firms will ID those bugs, looking for BCC. If purified water was a raw material in a non-sterile, aqueous product, that would be a good place to look for contamination. The focus of the chapter is on the final product but if your purified water is a big component of that final product, it seems smart to monitor for BCC unless there's some mitigating factor.

I can't speak on the second half of your question but perhaps some MFG can offer their input.

A28: As a sponsor of several contract manufacturers we required all oral and nasal liquid products that use PW as an ingredient to be tested for Bcc in finished product. We also instituted Bcc confirmation testing of PW for any gram negative organisms recovered during routine testing.

A28: I do agree that you should be testing your aqueous products for the presence or absence of Bcc even before USP Chapter 60 came into existence. I also agree that inhalation products be tested for the presence or absence of Bcc if they are aqueous or non-aqueous.

For your purified water, are you following the USP Chapter 60 to detect the presence of Bcc by doing an enrichment? In the past, most companies would perform Gram-stains on representative colonies on their water recovery plate counts. If a Gram-negative bacilli was detected by Gram staining, it would be identified to the genus/species level.

With USP Chapter 60 becoming effective in November 2019, it would be interesting to see if companies are now conducting enrichment testing on their purified water samples because USP Chapter 1231 has

not been revised yet to say that purified water needs to be tested for Bcc by enrichment. However, it should be noted that USP Chapter 1231 is a guidance chapter and USP Chapter 60 is not.

Furthermore, it would be interesting to hear whether companies are being questioned by the FDA during inspections if they are testing their purified water samples for Bcc by conducting enrichment. However, I do not think that many FDA inspections of facilities are occurring at this time to provide a sufficient answer with the current pandemic situation. However, I do believe that companies should be aware of this potential point of contention during an inspection especially if they say that they are generating USP Purified Water.

A29: From my experience Bcc testing is an expectation (not a requirement) that is being adopted by agencies, particularly for PW systems that are difficult to sanitize (e.g. PVC systems).

A30: Can you tell me if you required all the manufacturers to start the testing of each product the day the USP60 Chapter became effective or was there lag time allowed before testing started?

A31: For those checking for absence of Bcc in your Purified Water, used in non-sterile Products:

1- Are you relying for recovery (if present) on the R2A plate? PIA?
2- Are you staining any recovery on R2A (even if below your limits) and IDing any Gram negatives.
3-In the lack of written regulations, What is the regulatory expectation during audits?

A32: We expected compliance with USP 60 on or soon after effective date (12-1-19) however, as of today, all are in compliance.

USP <62> Suitability Inoculum level for validating alternative methods <1223>

USP <62> Suitability test states the inoculum level requires no more than 100cfu. (Use a number of microorganisms equivalent to not more than 100 cfu in the inoculated test preparation) What about the requirement on validating an alternative rapid method ? What's the practice being used in other companies?

A1: If you are going to validate an alternative or rapid method by performing suitability testing, I would think that you would also use an inoculum level between 10 and 100 CFU for each test organism.

A2: Depending on the application, product, rapid technology, method, matrix, etc, customers have tried to go to extinction <0.1 all the way up to 100 CFU and anywhere in between. As an example, we showed linearity and range with our Milliflex Quantum that showed equivalence down to 1 CFU all the way up to 100 CFU. The key is to show equivalence to your current USP 62 method.

A3: Yes, you can justify using less than 100 cfu during method suitability when validating an alternative method. Refer to USP 1223, Ph. 5.1.6, PDA TR#33 and the validation tutorials at http://rapidmicromethods.com.

VHP cycles for Manufacturing Isolator

This is regarding the VHP cycles for Manufacturing Isolator.

Vaporized hydrogen peroxide(VHP) is a sterilant used to decontaminate the internal surface of isolators. We run the VHP cycle for every product along with CIP(Cleaning in process) and SIP (Sterilization in process) as per the industry practice. However, we wish to obtain clarification from the forum on the following question.

If we have 2 or more campaign batches of the same product, Do we have to run the VHP cycle for every batch even though we don't have to open the isolator to change any change over parts and also for CIP and SIP handle aseptically?

Also so the same if validated during process simulation study.

We would like to obtain the forum recommendation and acceptance criteria if there any regarding the query mentioned above?

A1: It is acceptable to run one VHP cycle and then fill multiple batches of the same product. It used to be very common to do this on early designs of filling line isolators when decontamination and aeration times were very long. On newer isolators, the cycle times are much shorter, so there is not as much incentive to do this unless you are filling relatively small batches. There are some filling lines that it is possible to change the vial size without breaking the aseptic condition, so campaign filling makes great sense in that case.

If you are campaigning batches, then your media fills must simulate your procedures including the total duration of campaign. The longer time you allow for a campaign, the longer you will need to run your media fills.

Using Bact/alert dual temperature in sterility testing

Kindly I need to ask about the use of Bact/alert dual T in releasing the sterile products instead of the traditional membrane filtration method of (USP-71), is it acceptable or no?

A1: The BacT Dual-T can be acceptable as an alternative method, if properly validated with your product material

A2: This technology has been widely accepted, used and validated. Most use a simple temperature for incubation based upon papers published showing a best case temperature of 32.5 +/- 2.5 deg C.

A3: Ali, Yes, following the completion of an appropriate validation (e.g., minimum time to detection, method suitability, equivalence). I am currently working with several clients developing rapid sterility testing validation protocols for the BacT. Please let me know if you need additional assistance.

A4: The following 2019 paper, which validated the BacT at 32.5 deg C required the use of SDA to detect mold. Comprehensive Evaluation of Compendial USP<71>, BacT/ Alert Dual-T, and Bactec FX for Detection of Product Sterility Testing Contaminants. https://jcm.asm.org/content/jcm/57/2/e01548-18.full.pdf

This from the paper's abstract: "BacT/Alert testing at 32.5°C paired with a supplemental SDA plate as an acceptable alternative to the compendial USP 71 method for product sterility testing."

The paper also did not take into account method suitability; only testing challenge organisms. Do you have another reference to support 32.5 deg C on its own?

A5: For the BacT a common citation in Warning Letters is validating the different sample sizes versus the compendial method.

A6: I am curious, since the ASM paper cited used iFA and iFN culture bottles with the BacTalert Dual-T, what would the results had been if they used iAST bottles, especially at 22.5C? The iAST are formulated very similar to TSA more for industry use, while iFA and iFN are somewhat more complex media for more clinical use. We know TSA is favorable for mold (That's been argued to death in EM)

A7: The BacT/ALERT DUAL-T can be used for the release of sterile products and is better suited for lower volume samples up to 10 mL (direct inoculation) rather than membrane filtration.

The harmonized chapters of the European Pharmacopoeia Ph.Eur. 2.6.1 “Sterility”, Japanese Pharmacopoeia JP 4.06 “Sterility Test” and the United States Pharmacopoeia USP <71> “Sterility Tests” describe both the membrane filtration method and the direct inoculation method to perform a sterility test, so both methods are acceptable.

There is a long history of the use of the BacT/ALERT technology in the pharmaceutical industry for In Process Testing or Final Product Release supported by different publications, and automated growth-based technology with 2 incubation temperatures is also now considered a compendial method as per the description in the European Pharmacopoeia in chapter Ph. Eur 2.6.27 “Microbiological Examination of Cell-based preparation” for the specific cell-based products.

A8: Do not know. The Plus bottles contain an extra component to assist with neutralization of antimicrobials. Recovery of mold using this media in the presence of the test sample would have to be validated.

plate transfer time

A factory has two production sites. Both sites are monitored with environmental plates. The media plates are all incubated at one site. How long can i hold environmental monitoring plates before transferring them to incubation. Is holding at room temperature for 12 to 24 hours alright. I do not wish to perform a holding study presently.

Sub-visible particulate matter test for colour injection.

Can anyone had experience of analysis of colour injection for sub visible particulate matter test,. Is there any guideline which speak about colour injection analysis for sub visible particulate matter.

Also is there mandatory to do Sub visible particulate matter test for injectable suspension. I think the suspension contains large particles which cannot be filtered if we use microscopic method second think if we analyse by using light obscuration method the sample block the sensor. Any suggestions are helpful.

A1: Although a colored solution might look quite dark to the naked eye, especially in a beaker, the capillary tube through which it passes is very narrow and the optical density is much reduced through the narrow sample field. The dimensions of the capillary tube is typically in the 500x700um region and so even quite large particles are able to transit, they will be counted and sized in the largest size available to the instrument (i.e. >125um, or >400um, etc.).

The particle size is tied to the refractive index difference observed by the instrument (a fixed parameter set in calibration, latex spheres in water) and so any real world variance in this difference will affect size, and therefore counting.

The other issue you mention, is suspension; optical particle counters saturate at relatively low levels (certainly above required USP788 testing), but suspensions may need either filter counts, or an alternative technology that is able to measure high concentrations, such as found in a suspension.

Hand sanitizer testing

What test would you recommend to evaluate the effectiveness of alcohol based hand sanitizer? Any references?

Would it be acceptable using strains from USP51 plus Bacillus ATCC6633 to determine the effectiveness? using 30 sec and 1 min intervals.

A1: The sporocidal activity of an alcohol hand sanitizer is not that good.

The formulation provided in the recent FDA Guidance for Industry included hydrogen peroxide essential to eliminate bacterial spores in the ingredients.

If you are marking the hand sanitizer in-house in response to the COVID-19 pandemic I would limit its use to non-classified areas and forgo any efficacy testing.

See FDA Guidance For Industry - Temporary policy for preparation of certain alcohol-based hand sanitizer products during the public health emergency March, 2020.

A2: Are you using an EPA registered product? All antimicrobials with label claims legally need to have EPA registration. EPA provides guidance on the required testing.

Bacillus spores thrive in alcohol.

A3: In the United States, antimicrobial hand washes and hand sanitizers are considered to be Over-the-Counter (OTC) drug products.

To demonstrate the effectiveness for this type of product, you must follow ASTM E1174-Standard Test Method for Evaluation of the Effectiveness of Health Care Personnel or Consumer Handwash Formulations.

If you want to make a claim that a handwash or hand sanitizer will kill x% of organisms in a certain time period, you must follow ASTM E2315 Standard Guide for Assessment of Antimicrobial Activity Using a Time Kill Procedure.

In reference to [name redacted] post, the US FDA does not allow companies to make anti-viral claims for these types of product formulations. However, there are several ASTM methods such as E1838 and E2011 that had been developed to determine if your product has anti-viral activity. In addition, there are suspensions tests in Europe that had been used to demonstrate viricidal activity for a product formulation. The recent FDA guidance in how to prepare an alcohol hand sanitizer is for firms that manufacturer alcohol for incorporation into an alcohol hand sanitizer. I would not recommend that you make your own hand sanitizers if you are not making a marketplace alcohol hand sanitizer by using this FDA guidance document.

A4: This is for industrial production and we want to claim the label of " kills 99.99% germs''.

A5: If a product is used on an inanimate hard surface, it is a disinfectant that needs to be registered with the E.P.A. Because a hand sanitizer is used on human skin, the FDA has responsibility. Most hand sanitizers regulated under an FDA OTC drug monograph.

A6: Hand sanitizers are regulated by the FDA as Over the Counter (OTC) drug products. (The EPA regulates surface disinfectants as antimicrobial pesticides.)

Hand sanitizers can be evaluated using in vitro methods such as Time Kill (ASTM 2315) and in vivo methods including ASTM 2755.

A7: Bacillus spores can be stored in Alcohol. They do not "thrive". There was a method for preparing spore solutions and storing them in Alcohol.

Long-Term Survival of *Bacillus* Spores in Alcohol and Identification of 90% Ethanol as Relatively More Spori/Bactericidal - Pious Thomas

<https://link.springer.com/article/10.1007/s00284-011-0040-0?shared-article-renderer#auth-1>

Current Microbiology <https://link.springer.com/journal/284> volume 64, pages130–139(2012)

A8: Unfortunately your company must subscribe to Current Microbiology to download a copy. AS an consultant I would need to pay. Boo!

A9: Bacillus spores survive quite well in alcohol. When manufacturing biological indicators, we routinely shipped spores in alcohol solutions as the solution inhibited vegetative growth and did not inhibit the spores.

A10: For hand sanitizers, do you use the same contact times as what is said at ASTM 2315 (15 seconds, 30 seconds and 1min)? If we want to evaluate antibacterial lotion or antiseptic lotion, what contact time would you suggest?

A11: It depends upon the active ingredient that is present in the product formulation. ASTM E2315 is a method for assessing the antimicrobial activity of a product by using a time-kill method in which you determine the percent of reduction in a specified time period. However, ASTM E2315 cannot be used by itself to justify that a product has antimicrobial activity by the Tentative OTC Drug Monograph for Antimicrobial Topical Product Formulations.

If your product contains alcohol as the active ingredient (e.g. 60=70% concentration) and used in a hand rub product formulation, you would use ASTM E2755 - Standard Test Method for Determining the Bacteria-Eliminating Effectiveness of Healthcare Personnel Hand Rub Formulations Using Hands of Adults

All other actives in hand sanitizers will need to be tested by ASTM E1174 - Standard Test Method for Evaluation of the Effectiveness of Health Care Personnel Handwash Formulations.

A12: If you follow EN1276 and EN1650 (European Standards) the obligatory contact time for hand disinfectants is 1 minute. You may test additional contact times (< 1 min) depending on the time the product remains on the hand.

A13: No alcohol base sanitiser will not effective on spore for the same you can challenge the vegetative cells only.

A14: The European EN 1275 and EN 1650 are quantitative suspension tests for bacteria and fungi that are very similar to ASTM E2315. While in Europe, test results from EN 1275 and EN 1650 might be sufficient to demonstrate antimicrobial activity for an antimicrobial product formulation, but it is not for the United States because these products are considered to be Over-the-Counter drug products and are regulated by the U.S. Food and Drug Administration. For the US, the first test to be conducted for an antimicrobial product formulation is either ASTM 1174 or ASTM E2755 depending upon whether the product contains alcohol for the demonstration of antimicrobial activity in simulated use for handwashing conditions. In most cases, a 30-second time-exposure is used, but a shorter time period could be also used if desired.

When it comes to the type of microbial tests that could be used for demonstrating antimicrobial activity or anything else, it is always important to have a understanding of the regulatory perspective for a country in which a product is going to be sold in. Otherwise, you might be wasting time and money by conducting inappropriate microbial testing.

Testing of API and raw materials

What is the expectation on the micro testing of these if used in non-sterile pharmaceuticals? at development/early clinical phases and then in routine manufacture. Is it a must do, recommended to do or no guidance and at your discretion.

Any experience or actual references to industry practice or more ideally regulatory documentation would be appreciated.

If you do decide that the API requires testing, i know that USP<61> allows to reduce the amount to be tested routinely from the 10g for small batch sizes, however how do you determine what amount you need to use for method suitability as when in full scale manufacture you may be at a level where you cant justify amounts below 10g.

Media Fill for a primary container change

For major changes including primary container change (e.g. stopper), a media fill qualification is typically required. We do have a media fill program where process simulation is conducted twice annually under a bracketing approach. Have you ever leveraged the existing bracketing approach that represents the worst case as a rationale for not having to perform a media fill for a major change?

water quality for cleaning - non-sterile manufacturing

The facility will be classified as Grade D, is it acceptable to use potable water to prepare the disinfectants used for facility cleaning (e.g floors, walls, benches) or does it need to be Purified water?

if you need to rinse any excess detergent build up can you again use potable water or does this need to be higher quality e.g. purified water?

We would be using purified water and 70% Denatured Ethanol for equipment and product contact part cleaning.

A1: For Class D areas when they had been classified in non-sterile manufacturing facilities, I have seen people use either USP purified water or tap water to make use dilutions of disinfectants. If you are using a cationic surfactant as a disinfectant, hard tap water will have an adverse effect on the antimicrobial activity. From my perspective, I like to use USP purified water to prepare disinfectant solutions.

To remove detergents from equipment surfaces, I have no issue in using tap or potable water for rinsing residues away. However, I would not recommend the rinsing of non-alcoholic disinfectant residues with tap or potable water. I always recommend the usage of USP purified water to rinse away residues of non-alcoholic disinfectants from equipment surfaces. There is no reason to rinse away alcohol residues since they evaporate from equipment surfaces.

I do have a question for you. Why are you preparing your own 70% alcohol disinfectant solution? I would think that it would be easier to purchase a 60 to 70% alcohol solution that has an E.P.A. disinfectant registration number.

A2: To clarify your question. We do not use these prepared disinfectant (non-alcoholic) for cleaning of the equipment due to possible corrosive effects. There is either a validated CIP or manual cleaning where (purchased) 70% DE is first used and then rinsed with PW.

Do you have any more detail on why hard tap water will have an adverse effect on the antimicrobial activity?

A3: The references in which hard water can affect the antimicrobial activity of cationic surfactant disinfectants which are quaternary ammonium compounds are as follows:

Davidson, P.M et al.
Antimicrobials in Food. 2005, 3rd edition, Taylor and Frances, Page 531

Dvorak, G.
Disinfection 101. 2008. The Center of Food Security and Public Health
www.cfsph.isstate.edu

Cationic Surfactants, Analytical and Biological Evaluation. Editors: Cross and Singer. 1994, Marcel Dekker, Inc. New York.

High level disinfectant

Does a liquid high level disinfectant need to be registered with EPA after receiving 501(K) clearance as medical device?

bbc identification

I am looking for an identification system like API or BBL for the identification of B.cepacia, B. cenocepacia and B. multivoras. Can you recommend me any?

A1: There are no biochemical identification strips or kits that can identify the individual members of the Bcc. They are only able to identify a member to the group or complex level such as Bcc. It is very difficult to separate the individual members of the Bcc by using biochemical tests. If you even use 16s rRNA sequencing, you only get to the group level and not at the individual species level. With MALDI-TOF, you can get about 80% of the members to the species level. The problem in identifying members of the Bcc to the species level is their biochemical and genetic similarity to one another. There had been more success with gene targets in separating the individual members of the complex into individual species.

A2: How do we differentiate individual species of Bcc specially ATCC culture due to their biochemical and genetic similarity to one another specially with MALDI-TOF. Most of the industry use Phenotypic identification (Vitek or Biolog) and it's a challenge to get species level identification.

Is it okay if we report as a group of organism and not at the individual species level?

A3: When developing the Bioballs with the different BCC we used numerous different methods but strong traceability and a certified reference material is a way to be certain.

A4: If you are only performing phenotypic identification of recovered isolates, I do not think that it should be a problem to report the identification of a recovered isolate as Burkholderia cepacia complex since all members of this complex would be considered to be objectionable in a product formulation.

A5: You can use API or vitek if you want to do identification of Burkholderia cepacia.

A6: Yes, the Biolog GEN III MicroPlate can identify these species. It has a database of over 1500 species and covers both gram-negative and gram-positive bacteria with a single microplate test kit.

A7: At Charles River Accugenix, we can separate out members of BCC by sequencing the recA gene. This is our ProSeq service. Also, in our experience, MALDI is not capable at speciating BCC. Additional info can be found in an APR article here: <https://www.criver.com/sites/default/files/resource-files/MS-Why-should-you-care-about-Burkholderia-cepacia.pdf>.

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Using two microbial identification systems

I have questions regarding on using two different microbial identification systems (MicroSEQ and MALDI-TOF). Do you perform the comparability test showing those two are identical (meaning regardless of the system, the same ID comes out) or accept the fact that they are different? If performing the comparability test, what is the acceptance limit? If you accept the fact they are different, who do you control the different identification results?

A1: There is no need to compare the methods. You should select organisms that are in the relevant database and demonstrate the new method intended to be used will give good IDs from an accuracy and precision standpoint, while using organisms that you absolutely know the ID.

For accuracy, you should be able to obtain a 90% correct ID.

For precision, you should be able to obtain 95% repeatability and 90% intermediate precision.

A2: USP <1113> Microbiological characterization, identification and strain typing states:

"Microbiological identification systems are based on different analytical methodologies, and limitations may be inherent to the method and/or arise from database limitations. Identification is accomplished by matching characteristics (genotypic and/or phenotypic) to an established standard (reference) organism such as a type strain. If a microorganism is not included in the database it will not be identified, so manufacturers should review the breadth of the database of the identification system they plan to use and its applicability to their needs. Users should consider which microbiological identification system(s) is (are) most applicable to their requirements. Bearing in mind both these limitations and the level of identification required (genus, species, strain), users also must select the appropriate technology to use in routine microbiological identification testing."

I would not supply an alternate method validation approach to microbial identification especially as there is not compendial test or a gold standard.

New Publication by R. Hutt and Don English

New article just published by me and Don English based on the November 2019 PMF talk: Testing of Purified Water, Raw Materials, In-Process Samples and Finished Non-Sterile Products, American Pharmaceutical Review, Volume 23, Issue 3, April 2020, pp 46-50.

A1: Please, in order to buy the article you published, would you mind giving me the exact name, DOI, etc.

A2: The article will be available soon online, free of charge. Just google American Pharmaceutical review and you can look at any issue you want. This April issue is not yet online, but should be there soon.

A3: American Pharmaceutical Review is a free publication for the industry. You can find them at http://www.americanpharmaceuticalreview.com/ and sign up for a subscription.

material transfer process

How are you handling the material transfer process of items from unclassified area to classified area, as well as from class C or B to a grade A laminar airflow cabinets (sterile production). What disinfectant and method do you use at each step, and do you use a sporicide and/or IPA/ethanol to disinfect the items? How do you handle possible disinfectant residues? What about items, like single use sterile materials, that are packed in paper and cannot be disinfected multiple times?

How are you planning to approach the upcoming changes in new GMP Annex 1 that introduces the need to have a validated list of items transferred in with the process and chemistry used to disinfect the items?

A1: When moving materials from a lower classification to a higher classification transfer disinfection will be done with a sporicide followed by a suitable alcohol based sanitizer (i.e. 70% IPA or 70% Ethanol) to remove the residue.

After completion of the laboratory disinfectant efficacy study you should demonstrate the disinfectants used are suitable under in use conditions in the manufacturing environment. Conditions such as

temperature, air changes, and technique of application may have an impact on the effectiveness of the disinfectant.

The approach we are taking is that only materials and equipment that have been included on an approved list, developed during validation of the transfer disinfection process, will be allowed to be transferred into the Grade A or Grade B area via an airlock or pass-through hatch. Equipment and materials (intended for use in the Grade A zone) will be protected when transiting through the Grade B cleanroom. Any unapproved items that require transfer will be pre-approved by QA as an exception. Appropriate risk assessment and mitigation measures will be applied and a specific disinfection and monitoring program approved by QA.

A2: What kind of validation approach did you take for the transfer disinfection process? Are you executing the validation by sampling (eg. swab samples) the materials and equipment on your approved list pre- and post disinfection (sporicidal and IPA/ethanol?)

Does anyone have a suggestion for the disinfection of single use sterile materials, that are packed in paper and cannot be disinfected multiple times? My concern is that the paper could become permeable after wetting, and could risk the sterility of the item inside the package. Do others see this as a risk and how have you overcome the issue of possible microbial contamination on the outer package versus not risking the integrity of the material inside the package by the disinfection process?

Recording of test results

I need to know what is the industry practice of reporting results when testing performed by Membrane filtration process.

A1: Microbial count can't be report in decimal numbers. It should be either <1 or complete number for ex. 1, 2, 3, etc.

A2: You shall report total cfu per 100mL that is total volume you filtered on membrane.

A3: We always report CFU in whole numbers. Exception is when writing high concentrations that involve exponents such as 1.5 x 10^7 CFU/mL.

A4: Report as 16 cfu/ 100 mL.

A5: You report the bioburden result in terms of the sample size in the specification. e.g., WFI not more than 10 cfg/100 mL or pre-sterile filtration bioburden NMT 10 cfg/100 mL.

Also it makes no sense to report fractions of a cfu.

A6: According to your question as below:

Membrane Filtration:
> Filter 100mL- Count is 16 cfu
> If we have to report count in cfu/mL- 0.16 cfu/mL
> Can we report cfu in decimal or it has always to be whole number as this
> reported as per calculation.
You can record the result as below:
16 cfu /100 mL
Means 0.16 cfu/mL we can record this result as 1 cfu /mL . Either record and prepare trend as per 100 mL calculation.

Regarding anaerobic monitoring in Fill finish and sterility testing area

Is there any guideline requirement for anaerobic monitoring in fill finish and sterility testing area?

Is there any risk assessment required for inclusion of anaerobic monitoring for routine monitoring?

A1: Do you make products that would be likely to have anaerobic growth, e.g., strict oxygen control, oil-based products?

A2: Per USP <1116>: "In general, monitoring for strict anaerobes is not performed, because these organisms are unlikely to survive in ambient air. However, micro-aerophilic organisms may be observed in aseptic processing. Should anoxic conditions exist or if investigations warrant (e.g., identification of these organisms in sterility testing facilities or Sterility Tests <71> results), monitoring for micro-aerophiles and organisms that grow under low-oxygen conditions may be warranted."

Basically, you're going to need very specific reasons why you'd need to perform anaerobic EM. Almost no company does.

USP <60> Testing on Non-Aqueous Inhalation Products

Can anyone tell me if they are performing USP <60> testing on Non-Aqueous Inhalation Products? We are getting ready to validate a microbial method for a new inhalation product and we were wondering if we should include the USP <60> tests for Burkholderia cepacia complex if there is no water in the formulation.

A1: Good question [name redacted]. What is the patient population?...ie asthma, etc.

A2: If the product formulation does not contain water making it an aqueous product formulation, there is no need to conduct USP Chapter 60 on this non-aqueous product formulation.

A3: The USP is very clear that the requirement for BCC testing is for aqueous, nonsterile products.

A4: The non-aqueous inhalation product is for asthmatics or patients suffering from COPD.

A5: In the past I worked with a company that made an inhaled dry powder product for cystic fibrosis patients and we tested the powder for B. cepacia. I imagine the same holds true now, particularly with the issuance of <60>. If the product is being inhaled, even if for non-CF products, it's the right thing to do (testing for Bcc).

A6: Yes, USP is clear, however if I were manufacturing the product I would feel more comfortable exceeding the regs and testing the powder for Bcc. I like to get a good night's sleep.

A7: I agree with you that sometimes you have to conduct testing on a product formulation no matter what is indicted in the USP to have that assurance that your product would not cause any harm to the patient or consumer even though the possibility of having the presence of a particular type of microbial contamination is remote. From my perspective, the presence of any type of a Gram-negative bacilli in an inhalation product would be unacceptable.

A8: You should also check the water activity aw and use USP<1112> guidelines..

A9: I couldn't agree more. In addition, CF patients are susceptible to gram positive microorganisms as well, such as Mycobacterium abscessus and Staph aureus. Stenotrophomonas maltophilia (gram negative) is also pathogenic for these patients.

Although it's remote that these microorganisms would be found in dry powders, I would test for them as an extra measure of safety.

A10: i think that this statement is making things unclear:

"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"

it says especially, so to me it is telling you that you need to do it for these products.

it also doesn't clarify if its a DPI or pMDI with propellant, but it singles out those for inhalation use from the aqueous preparation. i interpret that as inhalation products need testing and not just the aqueous products.

A11: I could not disagree with both of. Gram negative microorganisms should never be allowed in the product category you both mentioned.

A12: It does state that USP 60 applies to inhaled products and the fact that your product is inhaled directly by COPD patients where Burkholderia could colonize and create a serious respiratory infection. In your case, I would certainly test for Bcc.

What steps should be checked if any Objectionable Microorganism is recovered in EM of a non-operation room?

I'd like to have a discussion about the actions upon recovery of objectionable organism in environmental monitoring. Please share your thoughts what steps should we take in such situation.

A1: I am not sure what a non-operation room is.

Over and above not exceeding the alert and action levels the identity of the EM isolates may be useful in determining the source of the isolates, e.g., water (R. picketti), personnel (S. aureus), packaging materials (B. subtitles), and walls and ceilings of the facility (fungi).

Setting requirement for the absence of objectionable microorganisms in Em may not be useful as term objectionable microorganisms applies to non-sterile drug products.

A2: Certainly, the organism should be subjected to disinfectant efficacy testing. From Parenteral Drug Association Technical Report No. 70, Fundamentals of Cleaning and Disinfection Programs for Aseptic Manufacturing Facilities: "Selection of organisms should be based on the type of environmental isolates recovered from the facility (environmental isolates are preferred); however, if facility isolates are not available ATCC cultures ... representing facility isolates are acceptable until facility isolates can be obtained."

A3: Water isolates are generally stressed due to the low nutrient conditions. If you use a high nutrient microbial growth media like SCDA as the initial recovery agar, it may inhibit the isolation of these nutrient stressed organisms giving a false negative test result. For this reason, it is normal practice to use R2A and Plate Count Agar that are low nutrient microbial growth agars to isolate organisms from purified water systems.

Regarding SCDA and R2A Media's

Why we have to use R2A media for water analysis. As well as SCDA for Environmental monitoring?

USP <71> neutralizer validation vs alternate sterility method

I am curious how far removed from the validated method in a USP General micro chapter you need to before you can really no longer reference the chapter, and instead would call it an "alternate method".

I have a product that needs a sterility method suitability performed. It is an antimicrobial suspension (not a true antibiotic, it's based on Bismuth), and I was sent some sterility suitability work that had been done by a different lab previously. They ended up using a modified D/E broth as the final growth medium (direct inoculation), instead of FTM and TSB. At that point, is it still even appropriate to reference USP <71>?

I haven't done any work with this yet so I'm hoping we can get something else to work, but I am wondering if we go down the same path, would this have to be fully "validated" as an alternate sterility method?

A1: I don't know of any customers using D/E Broth in a classical USP 71 method. That chapter specifically designates TSB and FTM as the media with the possibility of adding neutralizers for penicillin or cephalosporin.

Can you dilute or neutralize the product prior to adding the media?

A2: Can the product be filtered?

A3: I haven't done any work yet with this product, I was just looking over documentation that bad been provided for previous suitability work. I do know that this is a suspension and the particle size is 1 - 3 microns, so that would rule out filtration.

I have been wondering if since the antimicrobial activity is based on Bismuth, if any neutralization steps used for mercury would be a good candidate? I am guessing it's the Sodium Thiosulfate in the D/E broth that is likely providing the most "neutralization".

A4: I would look to see if a filter clogs up and if it does not, run some method suitability studies. You may be surprised if it works.

If not, I would recommend adding similar neutralizers (at the same concentrations) found in D/E broth to TSB and FTM and see if you can pass method suitability with direct inoculation.

This would be a good start.

A5: I think I will take this approach first and see how it filters.

A6: [name redacted] is quite right. In terms of recovery and equivalency to (71) D/R broth would be a poor choice.

A7: There is also sterile IPM as a solvent option.

Chemical Sanitization of Water System

I'm trying to find out what other companies are doing regarding the frequency of sanitizing the water loop. Specifically an ambient DI/RO system which feeds into a Hot DI/WFI system.
We are suggesting, as a quality unit, quarterly but being offered every 6 months.
If you can answer in terms of chemical sanitization that would be appreciated.

A1: For our ambient DI/RO system in plastic, we chemically sanitize on a monthly basis. There are no points of use on this system and it's to maintain the quality of a downstream purified water system in stainless steel. For that system, we use heat monthly and chemical annually.

PET test

if we have a single use non-sterile nasal spray that is preserved, does it require PET to be performed or is this only needed for multi dose product as this can be contaminated by patient during routine use?

A1: Single use does not require PET.

A2: Adding a preservative system to a single-use nasal spray would need to be justified to the FDA and when justified pass the USP <51> Antimicrobial Effectiveness Test.

Do you share the same formulation with a multiple use products? Is that the reason?

A3: If the formulation is used in a product package that is used in a single application, there is no need to conduct USP Chapter 51 testing. If the same formulation is used in a product package that is multi dose, challenge testing would be required on that product formulation. With a single use product formulation, you need to justify the inclusion of a preservative and it cannot be used to take care of any manufacturing issues.

A4: A single use product will need preservative since it is non-sterile. Challenge test can be performed at time 0 but not required over stability. Time 0 PET/AET will ensure that if you start with microbial counts less than specification, they will not grow over the shelf life. Microbial limits test at the time of release will ensure you have absence of objectionables and counts are less that the established specification. Presence of effective preservative will ensure the counts will not grow over shelf-life.

If you want to avoid putting preservative altogether, individual single use blow fill vials is an option (e.g., single use, preservative free eye drops).

A5: This would be a new developed product for a single use that has a preservative as part of formulation, not from an existing multi use.

Why would it need to be justified with FDA, and if AET is required is this just as part of development work or for routine stability assessment?

A6: We have tested many single use products, even ones that are non-aqueous, because of the FDA's request to test anything with a preservative in it, single-use or multi-use. Their justification when asked, seems to be from these two statements from the intro to USP <51>:

"Antimicrobial preservatives are substances added to aqueous pharmaceutical products. Nonsterile dosage forms may have preservatives added to protect them from growth of microorganisms inadvertently introduced during or subsequent to the manufacturing process."

and

"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."

The two sections, " Nonsterile dosage forms may have preservatives added to protect them....." and " Antimicrobial effectiveness, whether inherent in the product or produced because of the addition of an antimicrobial preservative, ... or for other products containing antimicrobial preservatives."

The second section does not mention single or multi-use nonsterile products with the statement "or for other products containing antimicrobial preservatives." This seems to be the statement that the agency is using for any product with a preservative in it.

A7: I do not understand as to why you are saying that a single use product will require a preservative and a preservative will ensure that the microbial counts will remain the same during shelf-life. If you are doing

this , I think that you have bigger issues in your manufacturing facility than just including a preservative in a single use product application.

From my perspective, you only add preservatives to multiple dose product formulations in order to prevent the proliferation of organisms that are introduced during consumer usage and not during manufacturing. With a single use product, you may have the introduction of organisms during consumer usage, but they will not proliferate because you no longer have any product left to use after the single application. I can see having a preservative in a single use product if that formulation is also going to be used in a multiple dose product package as a different SKU.

Your justification for including a preservative in a product formulation to prevent the proliferation of organisms during the product shelf-life is a little hard for me to understand. In general, the only way in which organisms could proliferate in a product formulation is that it contains a raw ingredient that could serve as a microbial nutrient. In general, the majority of raw ingredients in a non-sterile product formulation are not able to serve as a microbial nutrient. If you had one, there are a lot of other factors that are involved for a organism to proliferate in a formulation after manufacturing such as pH and the presence of oxygen. From conducting development work, I would think that you would see this before the product ever goes into production.

A8: Water as a major raw ingredient is not sterile. For non sterile products, even packaging components are not sterile. The manufacturing and filling is not sterile.

This is my opinion based on years of microbiology training and experience and not necessarily the position of my current or former employer.

A9: You're likely to be asked how this nasal spray remains unadulterated over the entire product shelf life. Non-sterile drug products have the potential to become adulterated through microbial growth or the presence of objectionable organisms in the finished product. Although this is a single dose product and not required to undergo AET as per USP <51>, preventing growth of the organisms inherently present in this non-sterile product and the exclusion or neutralization of objectionable organisms still needs to be addressed. Some options are:

1. Sterilize it and maintain container closure integrity over the product shelf life - this is costly and isn't required for nasal sprays but it solves the problem.
2. Add a preservative and show preservative effectiveness over the product shelf life (testing for preservative content may be adequate if the content is correlated to effectiveness). As Tony mentioned, adding a preservative to a single dose product may be frowned upon.
3. Demonstrate that the product, due to its formulation or nature (e.g. low water activity, extreme pH, high concentration of solvents), is not capable of supporting microbial growth over the product shelf life.

Each potential solution should be supported with data. If the product contains a preservative and the presence of the preservative has been accepted by regulatory authorities for a single dose product, demonstrating preservative effectiveness through the stability period may be the best solution for demonstrating microbiological control of the product.

A10: Nasal sprays are >90% water and likely to contain humectant such as CMC or Xanthan Gum, which will carry low level bio-burden. If making it sterile and blow fill in single use vials with snap off cap is not an option, adding preservative and ensure its activity (or content testing if correlated to effectiveness) to support shelf life is a good scientific approach.

Training model like PDA TR 35

In my company we used to make training models in line with PDA Technical Report No. 35 (2001) A Proposed Training Model for the Microbiological Function in the Pharmaceutical Industry. It was a valuable resource.

It is a retired document and the reason I found on the PDA website is that "it is no longer considered to represent current industry best practices".

I am not keen to present content which refers to the retired document, should I say outdated.

Is there any other similar document which can aid us in the training program, what would you suggest? I for one liked this TR very much.

Performing AET (volume to test and container)

if the product is ~200ul contained within an assembled device how do you perform the AET test?

The device will need to be completely disassembled to get to the internal liquid (200ul) contained within a small vial in the center of the device.

I'm assuming that this volume is too small to perform testing on as the inoculum volume of each organism will completely dilute the formulation.

What volume do you need to use and how do you justify that or do you just need to show that your inoculum is between 1.0x10^5 to 1.0x10^6 per ml of product solution?

Do you need to have representative containers manufactured to be the same material as what is in contact with the solution within the device?

What about any closure/stopper in the device as this is small and specifically tooled to fit and probably not able to get a larger one made for the purpose of AET testing?

A1: [name redacted] with a 200 uL volume I assume that the product is a single-use dosage so why would it be formulated with a antimicrobial preservative system?

If you want to conduct the AET you can use a larger volume of the formulation in a suitable sized container.

A2: This product has been transferred so i am dealing with previous decisions which is why in questioning certain aspects they have done. It is a single use preserved spray.

is there any guidance on what volumes are acceptable and container/closure needing to be the same as the ones in the device to ensure the same product interactions?

Burkholderia cepacia question

With USP <60> there has been a great deal of discussion about Bcc. One of the swabbing procedures we use in our plant has an enrichment step. Does anyone have any comment regarding how competitive Bcc is in a non-selective enrichment. We are a non-sterile consumer product manufacturing facility and find fast growing aerobic spore formers (Bacillus spp) in the enrichments. Has anyone used the Bcc medium as a broth to select for Bcc during enrichment?

A1: The Gentamicin and Vancomycin in BCSA post-enrichment should effectively inhibit Bacillus.

Monitoring qualification

We qualify our workers for environmental monitoring in Class C areas by letting them perform settling/ active/contact plates in parallel with an experienced worker.

What difference in plate count can i allow between the experienced and new worker and still pass the qualification?

A1: As it largely impossible to show an equivalency of results with environmental monitoring unless the sampler in training grossly contaminates the plates this type of procedure is misguided.

A2: For environmental monitoring you cannot compare the results between samples, there is too much expected variability. You can compare their ability to arrive at the same count when they read the same plates. Depending on the expected/normal results in the area you may need a sliding scale to compare.

Microbiologist qualification

Please give your inputs on qualifying analyst or Microbiologist on expired sample. If usage of expired sample is acceptable to regulatory bodies. If there is regulatory reference.

A1: Whether microbiologist or analytical chemist, the analyst qualification should include some side by side testing on same sample or spiked placebo to get desired recovery. You may use expired sample for this activity as well. Whatever your practice may be, it should be very clearly stated in your training SOP and make sure that the entire activity is recorded in training notebook or training instance of eLN. If you do use actual sample in parallel to testing by qualified analyst, your SOP should be clear that the results generated by qualified analyst will be considered valid. If it is a solid product and you do end up getting very different result, you should investigate if that was because of the inexperienced analyst making error or true results. You have to rule out if got different results because the contamination was not uniform or your product had content uniformity issues. To avoid this- companies use expired products or spiked placebos.

As far as I recall, there is no regulatory reference.

Microbiological water analysis

Could you give me your knowledge/opinion on the below matter?

Microbiological water analysis, in pharmaceutical company, is performed in duplicate for each sample. What is the best (allowable) Standard deviation/RSD between the two results from the duplicate testing? Is there any guideline to follow? If not, can you set your own RSD as per USP <1225>?

A1: In most pharmaceutical manufacturing plants, microbial testing of purified water is conducted by using membrane filtration in which a 100-ml aliquot is used. There is no need to worry about standard deviation between plates since a single membrane filter is used.

In 9215B. Pour Plate Method of Standard Methods for the Examination of Water and Wastewater, I do not see anything about plating duplicate 1.0 ml and 0.1 ml aliquots of a water sample from an undiluted sample and a 1.0-ml aliquot from a 1:100 dilution.

If you want to perform duplicate plate counts, I have no objections. However, see Table 9020 VI of Section 9020 for the acceptable range of an allowable logarithm difference for duplicate counts.

If you have a properly designed purified water system, the microbial counts in a distribution loop are normally low such as between <1 and 10 CFU/ml. I would think hat it would be somewhat difficult to establish an allowable standard of deviation for 2 plates at a sampling site with such low counts.

A2: With pharmaceutical grade water, the standard practice is to use the membrane filtration method where you use a single plate. No issue there!

A3: We currently use duplicate testing (2 plates) with the membrane filtration. In order to switch from the duplicate to single testing (1 plate), we have to justify this change. How can I justify it? I am thinking if I prove that between the 2 plates there is a good RSD it will be accepted by the inspectors. But, I couldn't find any source of RSD in microbial counts.

A4: The justification is to comply with standard water testing methods, i.e. AWWA/APHA standards as recommended in USP <1031>.

BET Test

Could you please tell about the BET of water insoluble products /RM? Should we prepare sample by dissolving this samples with its suitable solvent before test?

A1: Yes, to perform BET you must get it to an aqueous state for testing. Then most likely dilute with water for BET or possibly a buffer until any interference has been overcome. You may have to do some experimentation to find the optimal procedure depending on the sample.

A2: There are several solutions to get potential sample endotoxin into an aqueous, BETestable solution that may include the following:
1. Dilute in water heavily already at the first step, i.e. >= 1/100. Most substances are miscible with water to some degree. You simply need to get below the solubility limit. If you start with "weak" dilution in water (<=1/10), you may face difficulties in generating homogeneous mixes.
2. Initially dilute in an amphiphilic liquid such as ethanol, 2-propanol or DMSO and follow up with water. Surfactants such as polysorbates can help alternatively, but bear some risk of masking endotoxin.
3. Extract endotoxin by solving your product in a lipophilic endotoxin-free liquid like oil (in case your product is not liquid anyway), adding water, mixing them vigorously, allowing the two phases to separate and testing the aqueous phase for endotoxin.
For options 1 and 2, a "soft" endotoxin spike right before testing will suffice. However, option 3 asks for "hard" spiking into the original lipophilic solution. In this case, solve reference or control standard endotoxin in DMSO beforehand.

INHIBITORY OF SOLID SELECTIVE MEDIA

I am having one doubt about Inhibitory Of selective media, As per guidelines we need to challenge at least 100 cfu for inhibitory test, but is there any written criteria that while performing inhibitory, the volume of culture suspension we are going to spread on plate should not more then 0.1 ml?

A1: Guidelines says use 10-100 CFU culture, volume doesn't matter, I think 0.1 is ideal for spread plate method.

A2: Please check European Pharmacopoeia 2.6.12., paragraph 4-5-4-2-2 "Surface-spread method"

A3: I have always had reservations about quantitative growth promotion tests for selective and diagnostic solid media that was never designed for microbial enumeration. This view was shared by my late colleague Scot Sutton who advocated serial streaking.

I agree with [name redacted] that 0.1 mL is a standard spread plate inoculum volume but up to 1 mL may be used if the plates are pre-dried.

A4: Inhibitory test is a qualitative test, inoculation of test organism using any method, that is streaking, swabbing, surface spread is acceptable. However in your sop, the method of testing should be indicated.

On a final note, your references can be gotten from standard methods such as USP etc and test books, etc.

A5: It's also called as Perpendicular Streaking Method, which indicates the efficacy or antagonistic property of the selective isolates against standard pathogen.

Compressed air

Is there any reference regarding the sampling time required for particulate sampling of compressed air?

A1: Although nothing specific regarding length of sample time, it is expected that the quality of air meet the grade of zone it is being used for. So a Grade A overlay would need to be certified by sampling 1 cubic meter (time is dependent on flow rate of instrument being used), Grade C would be a 1 minute sample.

For routine testing on a daily / weekly cycle I would take 3 x 1 minute samples and use the average as final result, if any of the 3 were to exceed the operational tolerances (defined through risk assessment, not just the certification table), then and additional 3 x 1 minutes sample to ensure the single result was an outlier and can be treated in much the same way as you would an outlier in environmental sampling.

A2: Compressed air should be treated as room air. Therefore, as per ISO 14644-1 (Part 1) you should sample a volume of one cubic meter air which corresponds to 1000 litres air, and thus 10 minutes sampling (with air sampler).

Method suitability for phase 1 - non-sterile product

During the early phases of product development and up to clinical trials where it is intended to test a product to <61> & <62> to what extent does the method need to be assessed/validated and suitability performed?

A1: My personal recommendation is that you create an SOP or protocol detailing phase specific method validation/suitability plan. You start with one iteration of method suitability to ensure the product can be adequately dispersed and you have adequate neutralization/recovery. Continue to build as the formulation gets finalized in advance stages of development.

The expectation would be that you have three iterations of method suitability on preferably three batches by pivotal trial or validation batch including the method suitability/transfer at the commercial release site lab. The method validation plan/protocol/SOP must be approved by your QA as well as regulatory affairs. Any changes in the proposal must be amended and included in CMC section to ensure traceability.

A2: As a contract lab I have seen a few different approaches:
1. The developer tests only the raw materials for <61> and <62> to ensure that the materials being used are "clean" microbiologically. This is because the early formulation process is often on a lab scale or small pilot scale process (so there may only be limited materials available or it is being formulated in a "non-GMP" environment).
2. When I have tested early development products (lab scale), I have found the occasional product contamination especially in aqueous formulations. These were almost always traced back to a screw-up on the lab scale formulation.
3. All of the early development stages have suitability performed and the <61> and <62> run. This seems to be limited to larger pharma groups.
Or
4. No testing until a "GMP" batch is manufactured.

A lot depends on doing a risk assessment on the material. Aqueous and high water activity materials (like chewable gummy products) are much more likely to have microbial issues than say solid compressed tablets so some companies will do micro depending on the risk.

My personal opinion is that at minimum, the raw materials used for the product development (regardless of how early a stage) should be tested for micro. Aqueous or at microbial risk products should also be tested.

Any other product that regulatory agencies might even have the slightest interest in should be tested as well.

A3: If you are releasing batches for clinicals based on USP 61/62 then you must follow method suitability as directed in each of these chapters. Michael

A4: The answer is dependent upon the microbiological risk of the non-sterile product that is the subject of clinical trials. FDA guidance documents for phase 1 clinical trials and phase 2/3 clinical trials don't require the submission extensive microbiological test results for non-sterile products. Products with low water activity generally aren't required to establish specifications until marketing, although the development of those specifications and supporting test methods during later clinical trials is prudent. Aqueous products prepared and held for extended periods of time prior to use in the clinical trial, even phase 1 trials, may require microbial enumeration and/or preservative effectiveness testing to ensure that the microbiological quality of the product is acceptable.

Use of Bacteriophages

In reading the trade journals today,I saw that a cosmetic company had introduced a bacteriophage product formulation to reset the skin microbiome and for the reduction of skin inflammation and redness.

The product is using a bacteriophage against Cutibacterium acnes. In reviewing the composition of the product, it is pretty much a simple composition in which Sodium anisate and Sodium levulinate are used as the preservatives.

From my Quality Control and regulatory background, I have some concerns. To reset the skin microbiome, isn't this a drug product than a cosmetic formulation? I'm curious as to whether it would be appropriate to determine the level of bacteriophages in the product at the time of Quality Control release and at the end of stability. From my experience with probiotic dietary supplements, I would determine the level at Quality Control release and at the end of stability.

I'm just curious in hearing the opinions of others on these 2 concerns that are present in this forum.

A1: If bacteriophage were so effective every mothers son would have products on the market for all bacterial ailments.

If bacteriophage were so good there would be no bacteria. In natural communities there is always a balance between parasite and host. Otherwise the parasite would die out. How effective is the phage at controlling the bacterium?

It may have a modest effect on Cutibactetium but I doubt if it would erradicate it. But would other organisms come to take the place of the bacterium?

I fully agree that the viability of the phage at the start and end of the shelf life needs to be detetmined. Plus the minimum effective dose (if there is an effect).

A2: if there is no medical claim (such as regenerates skin cells or improves blood flow) it should not fall under the auspices of a drug. If it only reduces redness, that is not a medical claim and should be acceptable.

A3: Sorry to sound so cynical but some of these products find there way into the market, making claims, (many of which are not supported with the scientific rigor demanded by licensed pharmaceutical manufacturers). Products like probitics, certain "super foods", treatments by less scrupulous stem cell clinics are often marketed in a similar way.

MET analysis incubation period reduction

I need guidance regarding microbiological alternative methods.

As you know incubation period for TAMC is 3-5 days and for TYMC 5-7 days during MET analysis.

Can we obtain results in shorter time with alternative methods? If yes, is there any reference about this study?

What should be our approach to perform a confirmatory/equability study validation? Does anyone have experience regarding this issue?

BET question

How to proceed in calculating the endotoxin limit for a combined injectable product (vitamin B complex) which consist of three components that have different endotoxin limits, should I average? Or take the one that represents the worst case?

A1: There are several different approaches.

* You could use the lower EL for all of them (like you said)
* A second option could be using the formula K/M as in EP 2.6.14 / UPS <85> for the DP (if lower of the point 1 – worst case approch)

There is in UPS <1085> a specific chapter for that: "Calculating Endotoxin Limits for Combination Products". I think you should take a look. Generally speaking, it looks like USP advice an approach like the second one.

A2: The combined potential endotoxin load of the 3 components cannot exceed the endotoxin limit of, typically but not always, 350 EU/hour. There are many variables concerning this calculation and could likely go on for paragraphs to cover just some of the potential cases.

I would highly recommend that you read Chapter 85 of the USP and the FDA Question and Answer Doc for BET.

https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-pyrogen-and-endotoxins-testing-questions-and-answers

Also there are two great publications concerning BET testing.

The Bacterial Endotoxins Test (A Practical Guide) Edited by Karen Zink McCullough

Endotoxins, LAL Testing and Depyrogenation, Third Edition, Edited by Kevin L. Williams

Both are easily found on the internet (Amazon usually has them)

Also this article may be helpful: https://www.acciusa.com/pdfs/whitepapers/EndotoxinLimits_SeanJH-PR17-012.pdf

You might also find some helpful information here: https://www.acciusa.com/tools-and-resources/educational-content/

A3: The books and articles that [name redacted] has suggested are pretty much invaluable. I have been involved in endotoxin testing for many years and still find myself turning to them for guidance on occasion. They would be a wise investment.

personnel monitoring

I would like to know if you take samples of nose and throat from personnel who works in iso 8 areas in non sterile production plants.

A1: Based on the status of the world today, I definitely would take the samples.

A2: Personnel monitoring is generally not conducted in non-sterile drug manufacturing areas.An exception may be ISO 8 areas used to manufacture inhalation drug products.

Was your intent to take nose and throat swabs to monitor for the bacterium S. aureus or the SARS-CoV-2 virus?

ISO Standards

Overview:
I am in the contracted medical device manufacturing industry and we sell in the USA and in other countries (hence why we are following ISO standards). We produce non-sterile device in this building which are then shipped to a contracted sterilizer and then shipped straight to the distribution center from the sterilizer chosen by our clients.

Problem Statement:
Our gowning rooms are completely separated by doors to a manufacturing space (manufacturing spaces are ISO Class 8 and ISO Class 7). I have seen USP 797 reference the demarcation line in these areas but have not been able to find the definition for the demarcation line in ISO standards.

Question:
Does anyone know if the demarcation line is applicable to ISO standards? What standard is it in?

Current Thought:
The demarcation line is not applicable to cleanrooms producing non-sterile products. As "USP 797 - applies to all pharmacies that produce compounded sterile preparations (CSPs) including those in, hospitals, retail settings, radio/nuclear pharmacies, ambulatory care centers and long-term care facilities as well as other environments such as chemotherapy units, hospital nursing stations, and operating rooms”.

A1: ISO 14644-4 provides requirements for clean room design. It does not specifically include information regarding LOD but rather instructs you how to design and build a clean room. Essentially each ISO classified area should be separated from each other based upon the structure, airflow, differential pressure and EM specifications. If your gowning rooms are classified as ISO 8 and your manufacturing area is ISO 7, both must meet the criteria specified in the document.

A2: I appreciate your response. All our cleanrooms meet the requirements of ISO 14644-4. I am fairly new to the company and a demarcation line has been in our gowning rooms since the design. I questioned it because I could not find any information on the demarcation line in the ISO standards we utilize. I just wanted to be sure I was not missing a standard. Sounds like it is un-needed and it slows down the flow of our gowning process as we have about 250 operators and the traffic can be high. The line was place in the center and a bottleneck occurred. I plan to move the line directly in front of doors leading into the manufacturing space (but still located in the gowning area). That way auditors can see it but it is not in the way.

Aseptic Sim for an additional fill volume

We recently performed an initial aseptic simulation for a 10mL fill. We have since decided to create a multi dose vial which is a 25mL fill in a larger vial. As this decision was made after we were done the first 3, we couldn’t bracket to begin with.

I am looking for your professional opinions on if we need to do 3 aseptic sims for the 25mL or can we do 25mL for our 6 month simulation representing both the 10 and 25mL fills.

A1: Depending on details of the container / closure systems (do you use the same stopper for both?) and filling parameters, it may be possible to add a single bracketing run with the new vial. Timing would depend on when you intend to start filling the 25ml vial for commercial production. I would strongly recommend that the 25ml vial be included in an aseptic simulation prior to using it for any commercial production. If your stopper is different, then 3 runs would be necessary.

Bioburden testing

It is not an uncommon practice in the pharmaceutical/Biotech industry to use just TSA incubated at 30 -35 degrees for the enumeration of both bacteria and fungi. I have seen this method verified in accordance with <61> to ensure it is an acceptable practice for both bacteria and fungi.

Since this practice does not follow the compendial method exactly, as it does not use SDA for Fungi, is verification of the method sufficient? Or does it need to be fully validated per <1223> as an alternative Microbiological method?

A1: USP General Test Methods like <61> are provided for referee testing of compendial articles, in this case microbial enumeration testing for non-sterile drug products and pharmaceutical ingredients with USP monograph requirements. When the test is referenced in a regulatory submission it becomes a release test.

The Total Aerobic Microbial Count methodology may be used for in-process testing after meeting the USP method suitability requirements and the growth promotion testing of individual batches of soybean-casein digest agar. As the method suitability and growth promotion requirements both include the ability to quantitatively recover both yeast and mold it may be considered a general microbiological growth medium.

The purpose of the Total Combined Yeast and Mold Count is to selectively enumerate fungi within a high background of bacteria. In fact, Sabouraud Dextrose Agar was originally developed to isolate dermatological fungal pathogens by using a medium with a high concentration of carbohydrate Dextrose at la ow pH to suppress the bacterial growth. As Bacillus spp. can grow on the medium, antibiotics are usually added to exclude these bacteria.

In downstream processing of solutions of proteinous material often held at refrigeration temperature, the most typical microbiota are Gram-negative, oxidase positive bacteria not yeast or mold. Under these circumstances, soybean-casein digest agar has a long history of use for monitoring in-process bioburden.

A2: This is very helpful. It seems that using TSA alone to enumerate fungi as well as bacteria on samples with a very low bacterial load makes it an acceptable practice based on the science. My question is more regulatory than science based. If bioburden is performed on a product with a very low bacterial load using TSA to enumerate both bacteria and fungi, is it considered a compendial method, since the USP/EP instruct to use SDA to enumerate fungi? I have seen cases where auditors claim that the use of TSA alone is an alternative to the compendial method which then requires a full validation per <1223> Validation of Alternative Microbiological Methods.

A3: You are correct. If a monograph chapter requires the use of TSA and SDA, and you want to only use TSA, then you would have to validate the new method using TSA is as good as the existing, pharmacopeia method.

A4: Using an official method for Total Aerobic Microbial Count found in USP <61> that meets the method suitability requirements for bioburden monitoring would not be considered an alternative method subjected to method validation as described in USP <1223>.

Since the NECC fungal outbreak regulators and industry alike have been more sensitive to fungal contamination but requiring the use of SDA for routine downstream bioburden monitoring based on the potential fungal risk is not useful.

A5: From a regulatory point you are testing for TAMC, total aerobic microbial count, so it's not really an alternative method. The method suitability uses Aspergillus and Candida as well as the three bacterial species, so you are showing your method will detect fungi if present.

High Potent/Toxic Compound Filling

Does anyone know the regulatory requirements for continuous monitoring in an isolator during the filling of toxic or high potency compounds? Our isolator vendor is telling us that you only need to monitor continuous total particulates and air viables before and after filling processes, but that you would not monitor during the actual filling because it would expose your monitoring equipment (specifically a Met One and MAS) to the drug compound. They recommend not monitoring during the actual filling; however, I am unable to find any regulatory guidance stating that is acceptable. In my experience, you would need to monitor throughout the filling process. Any help or guidance would be greatly appreciated!

A1: The recommendation from the Isolator vendor makes sense. The revised Annex 1 in Section 9.20 states:

In the case where contaminants are present due to the processes involved and would potentially damage the particle counter or present a hazard (e.g. live organisms, powdery products and radiation hazards), the frequency and strategy employed should be such as to assure the environmental classification both prior to and post exposure to the risk. An increase in viable particle monitoring should be considered to ensure comprehensive monitoring of the process. Additionally, monitoring should be performed during simulated operations. Such operations should be performed at appropriate intervals. The approach should be defined in the CCS.

A2: That sounds suspicious to me. Clean the equipment afterwards and there should be no problem.

A3: This sounds appropriate to me and I've seen this approach used for iso 8 antibiotic manufacture when the active is fine powder which generated particles. The monitoring is designed to show that the area is controlled to the right level before the operation takes place and that it recovers within the expect time after.

However I would suggest that at this stage you look to some engineering solution to make the transfer a closed system which reduces the risk entirely. I would imagine that this compound will not just affect the monitoring equipment but the isolator will also be exposed to the compound. Even if it was dedicated , cleaning val would be challenging.

A4: I agree, monitoring for particles when the product is a fine powder does not make any sense. However monitoring during set up does make sense and of course cleaning after processing must verify that the product has been removed from all surfaces.

AET failures associated with acceptable preservative levels and associated conditions

I am looking for some published papers/article references on the following.

I have reviewed some previous PMF thread responses relating to the AET and how preservative assay being acceptable but there is failure in AET. tTere were quite a few responses of peoples experiences however i would like something to refer to. all help much appreciated.

Some responses were:

Sometimes that the preservative challenge tests from an accelerated aged sample does not predict what happens for a product formulation during real-time stability testing.

It can be seen that the acceptable level of the preservative is assayed in the formulation however found to be inadequately preserved. Preservatives could bind to other ingredients and no longer available to provide antimicrobial activity to the formulation. potentially the preservatives may become enclosed in micelles and they are no longer available to provide antimicrobial activity in a product formulation.

Accelerated stability conditions cannot always predict how the product will hold up at end of shelf life at room temp. The preservative content still comes in at 100% or other acceptable level on stability assay, but it doesn't pass AET even though it was working at T=0.

A freshly-made batch at lower percentage of label claim did not perform the same (AET results) as room temperature stability samples that reached the same percentage of label claim at the end of shelf life. For this reason, the chemical assay for stability samples did not correlate with the expected AET results.

A1: I may have contributed to this discussion. I am not aware of published papers but more likely the comments came from personal experience.

A2: In a book called Drug and Cosmetic Microbiology edited by Don Orth, I had written a chapter indicating in what could cause a preservative challenge test failure such as incompatible raw ingredients, preservative absorbers, solubility of preservatives, ingredient order of addition to a formulation during compounding, preservative binders, and pH. I had listed scientific references for each of these things that could inactivate or interfere with the antimicrobial activity of a preservative.

I believe that this book can be obtained on the internet. I also seen this chapter also on the internet.

A3: A good published reference for preservative effectiveness is the three part series published in American Pharmaceutical Review:

American Pharmaceutical Review, August 2017, Antimicrobial Preservatives Part One: Choosing a Preservative System
American Pharmaceutical Review, October 2017, Antimicrobial Preservatives Part Two: Choosing a Preservative
American Pharmaceutical Review, December 2017, Antimicrobial Preservatives Part Three: Challenges Facing Preservative Systems

There is a discussion about the interaction of preservatives with other excipients that may be what you are looking for. For example, in Part Two you will find this text:

"Such interactions may not involve conventional chemical transformations, but include more subtle binding phenomena e.g. hydrogen bonding, aggregation and complex formation. Although the overall level of preservative in the product may not change (as measured by chemical analysis) the preservatives may be bound to such excipients and not available in the "free" form; efficacy may be reduced. Determination of preservative efficacy is therefore mandated."

Give it a read and see if it has more of the information you are looking for.

Antimicrobial efficacy test <51>

Does the antimicrobial efficacy test <51> need to be done during product development? Or is it taken as a criterion for batch to batch release? Where can I find this premise in detail?

A1: Helga the AET is used in formulation development and stability studies of the first batches through expiration not product release.

A2: USP Chapter 51 should not be used as a routine Quality Control release test. For Quality Control testing of production batches, you can do either qualitative confirmation of preservatives in a batch or to fo quantification of the preservative levels to confirm the presence of preservatives.

USP Chapter 51 is done during product development and during real-time stability testing of production batches for confirmation of shelf-life.

A3: The AET is not meant to be a release test. It is performed during formulation development and on stability to ensure the formulation has suitable antimicrobial properties through expiry.

Performing AET

Hi All, i have some questions that may have differing opinions on expectaions as i cant see these activities mentioned within the chapter but should you be applying the principles within other microbiology chapters.

Questions.

1. Appart from proving neutralisation and acceptable recovery rates what other type of method suitability is needed before being satisfied that the method works?
2. do you need to prove suitability on 3 differnet batches?
3. do you need to use additional organisms from those 5 listed?
4. do you need to run a negative control at the same time? (either during suitability or in routine) e.g product neutralisation, dilution, plating without being spiked?
5. what do you do if you isolate some colonies that are not the expectation from what you inoculated? do you count and investigate or not?

A1: 1. No. Method suitability recovery rates should be all you need with a caveat. I have seen acceptable recovery rates in platings with distinctly inhibited colonies (especially with A. brasiliensis). For example, the 1:10 dilution may show a recovery rate of 89% and the 1:100 dilution has a recovery 82% for an organism. However the colonies on the 1:10 plating are obviously inhibited in growth (smaller/no spore formation/obvious morphological changes from the + control). I would still enumerate them with a note describing the inhibitory characteristics and use the 1:100 dilution.
2. USP <1227>, notes this:
"Recovery on Agar Medium
In the tests under 〈51〉 and 〈61〉, (USP 1-Dec-2019) the number of viable challenge microorganisms in the product is estimated (USP 1-Dec-2019) by calculating the concentration of cfu per milliliter by the plate count method. A design for validating neutralization would incorporate the treatment groups as described under Validation of Neutralization Methods—Recovery Comparisons. At least three independent replicates of the experiment should be performed, and each should demonstrate ▲a mean count of any of the test organisms not differing by a factor greater than 2, i.e., 50%–200% recovery, from the value of the control in the absence of product. If it is necessary to solubilize the test ▲sample,▲ (ERR 1-Dec-2019) the effects of the solubilization method on viable microorganisms must be determined. This situation can occur when testing ointments, suspensions, or other articles.▲ (USP 1-Dec-2019)"
Chapter <51> is silent on this however. Three seems to be the most appropriate.
3. "Semi-officially", B. cepacia seems to be the most requested additional microorganism, however, if you have had a product failure with any microorganism, I would absolutely add that bug to the list. I also see some common environmental isolates added occasionally (be careful of adding Gram + rod sporeformers however). These would typically be objectionable microorganisms. USP <51> notes this:
"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."
4. Negative controls are a great idea, especially in view of number 5 and also if the product has a lot of undissolved material in it.
5. Since these products are non-sterile, it is to be expected that there will be the occasional isolate that is not the inoculated microorganism. I would not count those. A good example would be having a recovery of bacteria as <1 log10 recovery at Day 14 (0, 0 cfu counts at 1:10 dilution) and a 1 log10 recovery at Day 28 (0,1 cfu count at 1:10 dilution of a microorganism that was not inoculated). This would fail for all 4 categories.

Incubation Time

I am having one confusion about incubation time. For Microbiology testing, we have criteria for incubation like 18-72 hrs incubation or 3-5 days or 5-7 days incubation. Now my question is, if i incubated a plate at 15:00 hrs of 12.06.2020 for 3-5 days, can I pull out the plate on 15.06.2020 at 12:00 (or before 15:00, technically not completed 3 days)?

A1: Please refer to USP <1117> at the end of the Microbiological Media Incubation Times section for the Pharmacopoeia expectation.

Generally I would think within 2 or 3 hours of that target time would be considered (generally speaking) the "same time of day" but this should be clear within your procedures and within your method validation.

A2: You want to ensure the plates are incubated for the full 3 days. The plates should not be removed before 15:00 on 15.06.2020.

A3: This is the guidance found in USP <1117> Microbiological Laboratory Best Practices:

MICROBIOLOGICAL MEDIA INCUBATION TIMES Incubation times for microbiological tests of less than 3 days' duration should be expressed in hours: e.g., “Incubate at 30° to 35° for 18 to 72 hours”. Tests longer than 72 hours' duration should be expressed in days: e.g., “Incubate at 30° to 35° for 3 to 5 days”. For incubation times expressed in hours, incubate for the minimum specified time, and exercise good microbiological judgment when exceeding the incubation time. For incubation times expressed in days, incubations started in the morning or afternoon should generally be concluded at that same time of day.

Low pressure/Low flow compressed gas sampling

I would like to know how others have handled the qualification of compressed gas systems where the point of use are typically <5 psi with flow rates <25 LPM. Specifically testing for viable air and total particulates. Traditional agar impaction techniques require a slightly higher flow rate to ensure proper impaction into the agar and total particulate devices, which use a gas diffuser tend to pull room air into the unit if flow rate too low. I'm potentially looking into using liquid impingement bottles (would love to know if there is a good SOP out there) for the viable air, and then considering using a 100L gas sampling bag to collected the compressed gas for the total particulate monitoring. Would greatly appreciate others insight into this.

Regarding Swab Surface Validation

I have one query regarding Swab Surface Validation:

I have to do Validation and after selected my area of sampling means the container from where I have to do validation.

Add culture on area... after that I perform sampling....take sample to lab and after doing analysis and analysis is from Pour Plate method.

Now I am confused abt temp condition...means in routine analysis we incubate our scda plate firstly into 20-25°C for nmt 3days for TFC then after 3days we transfer that plate into 30-35°C for nmt 2days for TBC....but when we do Validation or we can say we incubate our plates along with standard culture we have to incubate plate firstly into 30-35°C for nmt 3days then 20-25°C for nmt 5days....so which process I have to do?

A1: I suggest you follow a laid down procedure for your validation. You can check USP or BP or FDA documents on validation.

Gram staining timing guidance

I am looking for guidance regarding the required time frame for performing gram staining post test completion. ISO 11737 states that bioburden characterization is to be performed; however, it does not provide a timing requirement for the completion of the characterization.

I know that gram staining should be performed on young cells and I have seen documents from various companies that require staining anywhere from 18 to 48 hours. Are there any standards that specify the timing or provide guidance (perhaps a TIR)?

For those of you that are or have been regulatory authority auditors, what are your expectations?

A1: Gram stains on bacterial isolates should be performed on cultures that are 18 to 24 hours in age. If a culture is older than 30 hours in age, I have seen errors in how the stains of the Gram stain are taken up by cells that could lead to a mistaken identification for certain bacterial species. I m talking about what is Gram-positive and what is Grsm-negative rather than cell morphology.

In most introductory courses in Microbiology that I had taught, I always had recommended that. gram stain be performed on a bacterial culture that are 18 to 24 hours in age. In addition, most Microbiology textbooks recommend that Gram staining be performed on bacterial cultures that are 18 to 24 hours in age. If people are performing Gram staining on bacterial cultures that are 48 hours in age, it is indication that the laboratory is not following proper microbiological techniques in conducting microbial testing of samples from my perspective which could result in a big regulatory issue.

A2: The ASM clinical procedures handbook, Gram Stain chapter, is a good standard. It states <24 hrs culture timing to get more accurate staining reactions and interpretation.

Standard for pharmaceutical manufacturing job positions

Do you know if there is a standard (i.e. ISO, ASTM, etc, etc) that describes job positions in pharmaceutical industry?

For example, for a quality control analyst for medications or for a production quality inspector? A standard that describes the minimum competencies, skills and knowledge for that job position?

A1: The US Federal GMP regulations 21 CFR 211.25 Personnel Qualifications (a) states " Each person engaged in the manufacture, processing, packing or holding of a drug product shall have education, training and experience or any combination thereof to enable that person to perform the assigned functions"

A company must have job descriptions for each GMP position and the names, CV and GMP training records of each employee would be maintained and available for inspection.

A2: [name redacted] is correct that 21 CFR 211.25 indicates that every person should have the education, training and experience or any combination thereof to enable that person to perform the assigned functions.

From my experience, I have never seen a standard from either ISO, ASTM or any other organization that describes job functions and responsibilities in the manufacturing of drug product formulations. A probable reason for why there is no standard for job descriptions is that job responsibilities for a position can vary from company to company based upon organizational structure, type of manufacturing (e.g. sterile vs. non-sterile) and assigned daily tasks

Job descriptions for positions that are involved in the manufacturing of drug products is usually the responsibility of the hiring manufacturer of the company to establish by writing. In these job descriptions, there are often paragraphs indicating educational and experience requirements for a person to hold that position and a description of daily responsibilities. In addition, it is also usually the responsibility of the hiring manager to establish a training record in the functions and tasks for each individual that they are responsible for supervising and to keep these training records up-to-date on a year basis.

A3: You have received excellent information from [names redacted]. Also look at the requirements in the USP. There are some excellent threads from the past that may give you some help on the PMF.

Please note in your job descriptions that you not only have USFDA concerns to meet, but you may also have other regulatory bodies that you must meet. From the name of your company, I will assume that you are not within the US. So please ensure to incorporate your labor standards requirements into your job description. You may need to include the amount of walking, standing, sitting that will occur. You may also need to include the amount of weight that should be carried by the individual. These labor standards are not to discriminate, but rather set the standards that you are seeking for your company. With standards today, you may expect a person to work 8 hours a day, 5, 6, or 7 days per week. You may expect to pay overtime for over a certain limit of hours.

My comment is only to think thoroughly through your job description: what are you testing and for whom? what local and international requirements exist - DEA, FDA, ISO, radioactive, etc. Ensure that your job description meets the requirements that you may find of a labor standard site or other google search. Think about the types of individuals you want to have work for you. Think about how much training that you wish to give these people when they come to work for you. Remember that most universities do not teach industrial nomenclature, like normality, etc.

A4: I really appreciate your feedback. In addition to my question and taking into account that each company has its own internal organization, will you agree with the idea of evaluating and establishing minimal competencies related to manufacturing processes in pharmaceutical companies?

For example, the person who is charge of the microbial counting, should at least know: to apply aseptic process techniques, to count correctly the UFCs, to validate growth media production, etc, etc

Specifically, to develop schemes for pharmaceutical manufacturing job positions for a body that certificates people under ISO 17024?

A5: With respect to manufacturing job descriptions there are 2 general ways to go about it. One is to state generalities about the expectations of what the GMP operators should have, i.e., appropriate education, training, & experience. This leaves it up to the firm to decide which employees can perform certain duties (as long as they can show the employee has an adequate combination of the 3).

The other way is to lay down a guidance document to specify what is needed for various GMP positions. This route that will lead ever increasing detail as to what must be met to do certain jobs. This eventually leads to full certification, and an accreditation service. Then the emphasis becomes taking courses to gain certifications outside of the company and only those with certifications may take on new positions. In this scenario I fear that employees with years of excellent experience or that for any reason would not be able

to gain certification might be overlooked. Manufacturing firms are incentivized to get the right people in the right jobs. Let's let them work it out and avoid more regulation.

A6: In regard to your idea of evaluating and establishing minimal competencies of positions related to manufacturing processes in pharmaceutical companies, I do not disagree. It would be helpful, but the design and implementation for such a program would be the problem

However, I'm not sure that ISO 17024:2012 would be applicable because it is my understanding that it contains requirements for a body to certify persons against specific requirements and includes the development and maintenance of a certification scheme for persons. I still think that it is the responsibility of the company to establish educational and experience requirements and job responsibilities for each and every position.

The problem comes down to what are the common requirements and job responsibilities for a position at one company to another company since many people today are wearing many different hats due to cost and head-count reductions.

For example, I was responsible at one time for supervising the R&D Microbiology Laboratory but was also responsible for overseeing the Quality Control Microbiology Laboratories at different manufacturing sites and Quality Assurance auditors on a world-wide basis. I found it to be very interesting and stressful at times, but I did learn a lot and had fun in overseeing the many different aspects of my position.

MELTING CULTURE MEDIA

I want to know what do you use for melting or preparing culture media? A heating block or a stove?

A1: Preparing and melting should be two different processes but It's possible to use a hot plate that can boil the media before autoclaving and melting it for pouring but this does increase your risk of burning upon re-melting. I used a water bath to melt the agar and there are also hot beads that people use for re-melting and that would be my recommendation.

A2: I've seen both heating blocks and stoves utilized. Heating blocks, in my opinion are easier to stir, heat, and observe at the same time. But, I've seen companies that make large amounts of media over stoves successfully.

A3: We had an autoclave cycle that worked nicely to melt it. But... Is anyone still using microwaves for this?

Water Filtration Bioburden

For those of you performing bioburden testing of waters using the filtration method, are you using a pre-wet, a rinse, or both a pre-wet and a rinse? If a study is performed showing equivalency with and without a pre-wet and/or rinse, it is acceptable to forego them?

A1: How much water are you testing? If it's 100mL a pre-wet isn't needed. If it's a small amount like 5mL, you can pre-wet with 10-20mL of sterile water/diluent. Pre-wetting is designed to minimize any binding of the article you're wishing to test but water isn't a problem.

A2: 300mL, 100mL, 10mL, and 1mL. (dependent on water type). The 10mL and 1mL samples are placed in approx. 90mL to disperse the sample more evenly.

A3: Also, if you are testing a small amount, you can add it to a sterile diluent (such as sterile water or Fluid A, etc.) prior to filtering the entire amount. You'd want to do a negative control of your diluent.

A4: It is my understanding that membrane filter composed of mixed cellulose fibers have a quick wetting time in which a pre-wetting might not have to be performed. Is this correct?

What is your opinion in about using hydrophilic membrane filters that are composed of PTTE or Nylon if there is a concern about binding of ingredients in an aqueous formulation to the filter? From conducting preservative challenge testing, I do know that Methylcellulose is able to bond to certain types of preservatives. If there was an issue, I think that this would come up in the performance of suitability testing of the formulation in which spiked microorganisms could not be recovered.

For conducting membrane filtration of purified water samples, I see no reason as to why a membrane filter composed of mixed cellulose fibers cannot be used on a routine basis without pre-wetting since a normal aliquot for testing is 100-mls.

The next question then comes up as whether it is best to use a 0.22 or 0.45 micron membrane filter for water testing. I have seen many opinions expressed during the years in which one is the best for usage.

A5: Personally, I wouldn't see a need for pre-wetting, given the article being tested in this example. Water should have no compatibility issues with your filter and given the fact that you would be testing a minimum of 100mL of water, you would be fine to test as is (no rinsing).

To answer [name redacted] question, 0.22um filters are designed for bacterial RETENTION (i.e. sterile filtration, etc.), while 0.45um filters are designed for bacterial RECOVERY.

This debate is an old one, however, diffusion of nutrients from the agar plate are inhibited by a 0.22um filter due to pore size. 0.45um gives the best recovery of microorganisms due to improved growth conditions on the surface of the membrane. Many studies show this (I know Millipore has performed dozens, if not more over the decades). Given the organisms we routinely recover in water systems and filterable bioburden samples, you would not expect any organism loss due to the larger pore size of the 0.45um. You can always demonstrate this in a lab study should there be any doubt.

A6: Yes, the standard mixed cellulose membrane will have a very quick wetting time. The reason we'd want to add some sterile diluent is so the organisms are countable. With just a few mLs all the colonies will be concentrated in the middle of the membrane and make it difficult to count.

We sell a PVDF filter that's designed to avoid binding antibiotics and other compounds. Yes, sometimes during method development you'll find a binding that you might not have been aware of and see some inhibition. We work with many customers who might be having this issue and moving to a PVDF filter is one of the options. We have a compatibility chart that's more based on chemistry but it's useful because it has many compounds that you'd be able to see upfront aren't compatible with that particular filter.

Yes, any sample over about 10mL is probably perfectly fine without a pre-wet. All we're trying to do is spread out the contamination, if present, so it's countable.

The .22u vs .45u argument is still prevalent although 95% of firms do use the .45u filter for bioburden testing. The biggest reason is that the pore size is nominal, meaning the largest pore would never exceed .45u but the average pore size is smaller. These larger pores allow the nutrients from the media to get to the colonies faster. "Not larger than .45u" is also written into the guidances, which I'm sure you know, so that's what people follow. We performed a study of .45u vs .22u and both were able to retain B. diminuta up to about 1000 CFU before any passage occurred in the .45u filter but the recovery on the .45u filter was about 20% higher with larger colonies. In addition. The .45u filters product faster that can also reduce the chances for binding product.

Decreasing the sampling frequency in Environmental Monitoring

Is it acceptable to decrease the sampling frequency as below table in an aseptic filling facility depending on the WHO Environmental Monitoring of Clean Rooms in *Vaccine *Manufacturing Facilities?

Classification	In operation (dynamic) routine particulate sampling
Grade A (filling operation)	For the full duration of operation
Grade B	Daily[1]
Grade C	Weekly
Grade D	Not required
UDAF work stations in B	Daily [(1)]
UDAF work stations in C	Weekly
UDAF work stations in D	Monthly
UDAF in UNC areas	Routine re-qualification of UDAF is sufficient

[(1)] Working days. Monitoring can be omitted on e.g., weekends if no production activities are taking place.

Kindly note that we currently following the USP and ISO 14644 standards and frequency.

neutralization of copper

Can anyone recommend a neutralizer for the antimicrobial properties of copper? I have seen DE and lecithin /polysorbate described, but wanted to check in with this group for further information. The copper product is a liquid and we will be testing it in suspension (time kill) , as well as a spray applied to a surface (hard surface disinfectant). We will be challenging the product with both bacteria and viruses, so we need to consider the toxicity of the viral host cells. Any info will be appreciated.

A1: Have you looked into EDTA solutions? There are a lot of papers discussion the chelating properties.

A2: Besides using a chelating agent such as Disodium EDTA that Rick had recommended, Trypticase Soy Broth plus Polysorbate 80 (1.5% v/v) and Lecithin (0. 07% v/v) had been used to inactivate the antimicrobial activity of copper in the following paper:

Montero, D.A. et. al. Antimicrobial properties of a novel copper-based composite coating with potential for use in healthcare facilities. Antimicrobial Resistance and Infection Control (2019) 8:3.

I remember doing fabric antimicrobial neutralization studies in which copper had been imbedded to control odor-causing bacteria for deodorizing studies and used Trypticase Soy Broth with 4% Polysorbate 20, 0.5% Soy Lecithin and 0.2% Disodium EDTA as the neutralizing broth.

MET Testing for MDI Canister Placebo

We are being asked to test the placebo of a product in an MDI canister for MET as part of the release spec. We do test these types of products, the only problem is for this placebo there is nothing in the formulation besides HFA. Once opened the HFA is going to evaporate leaving you with nothing but an empty canister. I am not sure what approach to take on this besides just doing a rinse of the inside of the canister? There's not really any guidance on something like this since there is no solid/liquid product to test and it's not a something you would test like a device.

I was curious if anyone has run across a scenario like this. The only other option I could think of would be to expel the HFA through a filter but I'm not sure if that would be the best approach.

Any one have experience to validated Water shower sterilizer through Biological Indicator

Anyone have experience to validated Water shower sterilizer through Biological Indicator?

We try to validated water shower sterilizer at 107°C for 180 minutes by using Geobacillus stearothermophillus & Bacillus subtillis but BI not passed.

Is any alternative BI to validated water shower sterilizer?

Or other procedure to Qualify water shower sterilizer ?

We want to sterilize LVP(Packing mode LDP) by using this water shower.

regarding settle plate exposure and active air sampling

Can anyone tell what is frequency of settle plate and active air sampling in Class D area for non sterile manufacturing units?

A1: Other than the EU Annex 13 requirement for conducting inhalant manufacturing in a Class D environment there is no air cleanliness standards mandated for non-sterile drug manufacturing. The frequency of monitoring should reflect the risk associated with the dosage form and may be weekly, monthly or quarterly.

I recommend you refer to USP <1115> Bioburden Control of Non-sterile Drug Substances and Products.

A2: You can reference USP 1115 for great information on microbial control in non-sterile MFG units.

A3: Could you tell me where is indicated the air monitoring for non sterile manufacturing in EU Annex 13? I'm referring to USP 1115, but I work in Italy, so a EU guidance is preferred for us.

A4: Quarterly.

rapid sterility

I remember reading a justification for sample size for sterility tests for small batch sizes for example in radiopharmaceuticals. It contained a calculation of what percent to use from the batch. I think it was in the USP. Can anyone help me find it?

A1: USP 1071

A2: I think you're looking for USP <823> Positron Emission Tomography Drugs for Compounding, Investigational, and Research Uses. Section 8 provides information on controls and acceptance criteria for finished products. It also references USP <71> as another sterility method that can be used.

A3: It was mentioned in USP chapter 71 for sterility test.

Storage of cultures and media

What is the industry practice for storage of media and cultures together. We are storing prepared media for 15 days in a cooling chamber 2-8 degrees and store working cultures on nutrient media in separate

refrigerator . Can they be stored together in separate trays in one equipment. All media is wrapped and all cultures remain intact in close test tubes.

A1: Although it may look better to segregate live cultures and media, in labs I have managed they have been successfully stored on separate in walk-in refrigerated rooms under the conditions you describe.

A2: Though separate refrigerators are recommended, you can store them in the same refrigerator with Media on top trays and cultures on bottom tray.

A3: I have seen people storing the microbiological cultures, including new vials, inside locked containers with numbered padlocks and the in-use cultures separately inside a locked container with a combination lock, along with prepared media in the same walk-in refrigerated rooms maintained at 2-8 degC.

A4: There are three issues we are dealing with here- segregation, cross contamination and security. While having dedicated "clean" and "dirty" refrigerators are ideal from the lab design and flow point of view, it's not practical and economical for each lab. Microorganisms are not going to jump from plates and slants or tubes to sterile media. Even the theory of top shelf versus bottom shelf does not sit right in my mind but if it helps with segregation, so be it. Clear segregation in baskets or closed containers and label is enough for GMP purpose. Think twice before making permanent labels on walls or shelves. I have seen working culture tubes on shelves marked as Culture Media and vice versa. Cleaning and sanitization frequency including the cooling fan cleaning is critical element of periodic maintenance and your best bet in avoiding gross aerial contaminations inside incubators and refrigerators.

If security is a concern- card key access to the lab, user log, locked doors with key pad access, etc. are various measures. Although I must say, never heard of padlock on a container in my 30 years in the industry. It just increases the time you have to spend in a walk in chamber and might cause more problem.

A5: You can keep your working cultures and media in the same refrigerators. We keep our cultures plates separated by placing inside a ziploc bags and put them inside a clean covered plastic shoebox. We keep fungal plates separate from bacterial plates in two different boxes.

Also, as you know, bacterial cultures does not become airborne. Fungal plates if kept closed inside a ziploc bag within a box, can not release spores in the refrigerator.

So, one can safely keep media and cultures in one place.

Capabilities

I’m working with a state university microbiology department to establish applied capabilities/experience for interested/selected micro students (BS) beyond the typical undergrad curriculum. For example and in context of PMF – we anticipate practical/theoretical training re. USP’s 51, 61, 62.

If you were looking at a BS level microbiologist fresh from school, what skills/capabilities would you think esp. valuable in your hiring decision?

A1: Interpersonal skills, such as the ability to get along with others, excellent verbal and written skills, ability to compromise. Specific technical skills can be taught through on the job training.

A2: Quality Control! They learn methods quickly, but media control, IQ,OQ,PQs, method suitability, calibrations, validations etc. are the difficult things to train because they have never seen them. Give your TA’s a break and have the students do some of the control work. I often look at Medical Technologists for incoming Microbiology and chemistry positions because they understand controls from their internships.

A3: Clearly in addition to basic microbiology, understanding of GMP, aseptic technique in a hood, isolator and cleanroom, regulations, writing and adhering to SOP's and all the reasons why would be at the top of my list. Understanding of the ramifications of data integrity errors and why "unimportant" details are

important everyday. Even a course on Pharmaceutical manufacturing and how QC/EM fits into that process.

A4: I would value knowledge and understanding of GMP generally and as it applies to the lab, knowledge and skill in Good Documentation Practices for the lab, understanding of method suitability, hands on experience with at least the direct plating and filtration methods for testing, understanding of quality control for materials.

A5: The approach I would take would be to inventory all the job requirements for the success of an entry level microbiologist in the pharmaceutical or cosmetic QC lab and determine if these are best taught in course work at the state university or in on-the-job training at the pharmaceutical company.

For example, the state university should provide a broad scientific education, some basic laboratory skills especially aseptic handling of microorganisms, plate counts, Gram stains, etc, encourage the ability of the student to think critically and express themselves orally and in writing. By looking at the subjects taken, the grades achieved and intrapersonal skills of the applicant, the hiring manager should be able to identify who to hire and make an investment in.

The pharma company should train the new hire in record keeping, GMP compliance, and the compendial test methods. The pharma company should emphasize their culture especially dedication to quality and teamwork associated with working in industry.

A6: It’s a big area but you are getting expert advice from the members.

My favorite area (and one that often crops up at audit) is control of the manufacture, storage and shelf life of reagents. Sometimes in a lab you find reagents in the cupboard that have been there for years, have no expiry date, are poorly or not labelled. Everything you use should be manufactured to a validated process, subject to a recognised quality system (3rd party suppliers audited). Reagents must be clearly labelled, have correct h&s symbols, date of manufacture, expiry date, location in the lab. Anything out of date should be disposed of correctly. It gives a great impression if all this is under control.

A7: Introduction to bioburden testing, USP Chapter <71> Sterility Testing, microbial identifications (both old school and automated). Gram staining is really important as is aseptic technique. At least some knowledge of what constitutes a medical device, a biologic, and a drug.

There is just a lot to know and if the microbiology graduate has some knowledge of different aspects supporting this industry it would go a long way for me as a hiring person.

A8: They need to know aseptic techniques as a starting point and growth promotion as the next step. Everything after that is "gravy".

A9: I must disagree with many opinions expressed by most of the respondents.

A course in general microbiology taught at a state university for students that may work in clinical, food, water microbiology as well as the pharmaceutical industry should include a basic knowledge of the principles of microbiology and as a skill set to work with microorganisms in a laboratory setting. The course in microbiology may

Topics like the development of drug products and their manufacturing processes, GMPs, record keeping, compendial tests are best learnt on the job.

A10: From my perspective, I think that it is important for students to have basic understanding in how to biochemically identify microorganisms such as bacteria and how to use 16s rRNA sequencing to do mold identification since identification of molds by looking at reproductive structures under a microscope is no longer taught to students.

In addition, I think that it is important for students to have a basic understanding of chemistry to understand how to add preservatives to a product formulation so that a product formulation is adequately preserved against microbial contamination during consumer usage.

Students should at least be aware what are cGMPs .

A11: I will add my suggestion… many good ideas about classes and skills have already been suggested. The one I will add is the development of critical thinking skills ... in my opinion the joy of doing science is not memorizing something and repeating it on an exam but applying and thinking about what one has learned.

A12: Interesting question. I assume, from what you are describing, that this course would be beyond the basic micro courses and more like a choice for a higher level course (what I knew as 200, 300 or 400 level courses). Therefore, assuming that they have had the basic chemistry and micro classes, I would recommend several topics (in no particular order):
1. Source of the regulations followed, and why (GMP's, History of FDA, general information about international regs (ISO?). It is scary how many people I have interviewed with 5 years or more of pharmaceutical micro experience, who don't know where the GMPS come from (SOPS?) and that they are regulations.
2. Role of FDA (inspections, approvals, 483/warning letters, etc.
3. Basic overviews of pharma manufacturing and how micro interacts.
4. Qualification of facilities, esp with focus on water systems.
5. Roles of other functions in pharma operations, like QA, Regulatory Sciences.
6. Introduction to microbial investigations.
7. Introduction go basic microbiology compendial tests (sterility, mlt, bioburden, etc, identification techniques)
8. Future stare of pharma micro, like Process Analytical Microbiology (Rapid Methods)

And this is just a start.

I hope that the person or persons who do the training actually has experience in pharma micro and will not present topics, such as GMP regs, with an attitude about them.

A13: I also agree with many of the points made here. I once came upon a statement and forgive me I'm sure I won't quote it correctly but as I remember the point "You hire a resume and you fire because they can't get along with anyone". I've been in labs most of my career. This is the most true statement I've come across concerning hiring lab techs. I've seen some many come and go. You can train a good person to do most anything as long as they continue to show promise. You can't train someone who thinks they know everything and they screw up the culture in the lab, everyone in the lab and the work in the lab suffers.

A14: I would just like to add that I found risk analysis a really valuable tool in my learning process and may have appreciated a much better understanding of it earlier in my career.

USP <62>

Is anyone currently using a rapid method for USP<62>?

A1: Yes, we are using biolumix for pathogen detection.

Ready to Use Materials

For ready to use materials (vials, syringes, stoppers, seals) for sterile products, is there any guidance on only performing CofA testing on some receipts vs. every received receipt? I am looking more to not

perform sterility/endo/dimensional testing on every single lot. Is it just a risk assessment that needs to be performed or certain amount of receipts tested per year?

Alert/action limits regarding cleaning validation studies

Could you please tell me your approach regarding setting alert/action limits for microbiological evaluation of cleaning validation studies?

Do you use same limits for sampling “before cleaning” and sampling “after cleaning”?

A1: I think that you are confusing equipment cleaning and equipment sanitization.

For equipment cleaning, most people will set either a Total Organic Carbon (TOC) or Conductivity limit for a deionized water rinse in which they are measuring the presence of product residues and cleaners on an equipment surface after completion of the cleaning process before equipment sanitization is conducted.

For equipment sanitization, most people will set a limit based upon the microbial test specifications for a finished product. Unlike air sampling and water sampling of a purified water system, I have not seen people set alert and action limits for the numerical levels of microorganisms that may be present on an equipment surface after equipment sanitization. If you are going to set alert and action limits for equipment surfaces after sanitization, you need to base it on data collected.

However, I do question the purpose for setting alert and action limits after an equipment surface has been sanitized. Instead of setting alert and action levels, most people will validate their cleaning and sanitization procedures for manufacturing equipment. I can see performing periodic monitoring of equipment surfaces to ensure that there is no drift in how cleaning and sanitization procedures are performed after validation of the cleaning and sanitization procedures for equipment.

Sampling device for metered dose inhalation canisters

USP <610> describes alternate sampling methods for inhalation and nasal products; under the "room temperature method" for high-content INDP it mentions using a ice maker water tap line or other type of needle apparatus to puncture the canister and expel the contents through a line, either into a vessel or directly into the broth/buffer media.

I was curious if anyone knows of any devices available for this specific purpose? We have automatic can cutters but that is an enclosed unit that cuts the entire top of the canister off, so that wouldn't work in this application. I'm looking for something that will allow controlled release of the MDI contents into a vessel.

A1: From my experience, I have always used the chilling method for sampling pressurized aerosol inhalation products in which the outside of the containers was disinfected first with sterile 60-70% alcohol and allowed to dry. After the outside of the aerosol containers were dried, they were placed into a dry ice bath or a low temperature freezer for freezing before the containers were punctured for conducting microbial sampling.

I always wonder about the ice maker water tap line that is present in USP Chapter 610. I think that the device that you are looking for from USP Chapter 610 is an actual ice maker water tap line connector that is used normally to connect a refrigerator ice maker to a copper water line within the house. By connecting a sterile plastic hose to the end of the ice maker water tap line connector that is used to deliver water to a refrigerator ice maker, you can control the flow of the contents of an aerosol container into a diluent or broth by puncturing a hole into the pressurized aerosol container by using the needle of an ice maker water tap connector and withdrawing the needle to control the flow out of the punctured container. These ice maker water tap line connectors are readily available at a hardware store.

I will admit that I have never seen a commercial sampling device with a needle for puncturing pressurized aerosol inhalation containers since I believe that the marketplace for such a sampling device would be exceedingly small.

A2: I am going to try exactly as you mentioned and buy a few of the tap kits from Home Depot and get some tubing that will fit. I figured it would be pretty easy to autoclave these in a steam sterilization pouch to take into a hood for testing.

limit of alert and out of trend

Do you investigate any single breach of alert levels or 3 consecutive breaches?
I work in a non sterile manufacturing (oral solid) and we want to review our procedure.

Could you suggest any guidance about alert levels in environmental and how to manage OOT?
What is your experience about it?

A1: I have always had treated 3 consecutive alert as an action limit. Just like an action limit, three consecutive alerts will require investigation an corrective actions (i.e. repairs, cleaning, resampling, etc). The practice has been found to be acceptable thru all my regulatory and customer audits as a non-sterile CMO manuf. Hope this helps.

A2: A question about the first/second exceeding in alert limit. Do you notify to other departure (QA, QP, manufacturer) the exceeding the alarm limit without preliminary investigation? We want to perform investigation as OOS after three consecutive alert limit but the guideline are not clear about it, someone want investigation for alert and action, some other only for action limit. For us a OOT is three consecutive exceeding in alert limit, not only one but I need more guidance to prove it and explain to audits.

A3: You can use text from PDA TR13 Fundamentals of an Environmental Monitoring Program to support investigation after three consecutive results that exceed alert level:

"Three or more consecutive points or drifts may be considered to be a pattern or cluster formation that, if above the alert level, signals a trend that requires investigation."

Also, USP <1231> for water systems mentions "Exceeding an Alert Level repeatedly" as something that should elevate to Action level status which should prompt "immediate notification of both QA staff and the personnel involved in water system operations and use".

A4: Not really under the procedure; however, it is advisable to let Quality and Engineering know in case you hit the third and an action is required so they are prepared.

For non-sterile applications there is really no requirement. Assuming you are a contract manufacturer, you have to set your practice and defend it. Also base it on risk, if you almost never exceed consecutive alerts then risk is low, maybe your hits are more true excursions. Otherwise, you may have a harder time convincing your auditors.

In the CMO business it is hard to make all customer clients happy but if you have your position supported by scientific rationale and risk assessment, you should be fine.

A5: We also are a non-sterile CMO manufacturer. We investigate every monitoring exceeding, if it is alert or action, by root-cause-analysis.

It is communicated to QA, manufacturing/packaging dept. & QP. We may define actions, but we only have to for action level exceeding. For 3 consecutive alert level exceedings, we act like action limit exceeded.

As to have a rationale for this, we defined the alert (calculated by historical data) & action (given by guideline) level as follows: The alert level is the highest mark for a normal/routinely working process (manufacturing, cleaning & disinfection), exceedings do not mean, we would have a risk to product, but the process did not work properly. The action limit, may - depending on the height and the type of testing (floor, ceilings, walls, personal, machines, etc.) - mean that there can be a risk to the product or a big fault in the process, which has to be clarified and CAPA defined for not undergoing such an event again.

non-sterile product - component parts testing and ass**embly**

A few questions related to componentry please:

1.If i have a nasal spray do i have to test the component parts that are primary packaging for the non-sterile product?
2. If i have a design where the product is filled into a small bottle with a stopper (Grade D cleanroom), however this bottle/stopper is then packaged into a device that acts as a nasal spray and when activated the product flows up through the nasal spray nozzle, does this additional packing step still need to be done in the Grade D area?

It seems to me that my product is contained in its primary packaging so its quality is protected, but it will flow through a device part that could add micro bioburden. I have though about other examples and in an inhaler you have a synchroner mouthpiece that you put in your mouth and wouldn't test this and wouldn't expect to have the packing line in a Grade D room. Its unlikely to maintain classification with these processes.

A1: Here is my suggestion

1. All the components coming in product contact required testing and conform to established specification (this includes TAMC, TYMC and absence of objectionable microorganisms)
2. If product delivery happens through additional connections, then YES it is required to under classified area to avoid microbial contamination during assembling and packaging.
3. You should perform final testing after assembling all device parts/pathway. This will provide quality results for your drug product including delivery device.

A2: I can offer up how nasal spray products are handled where I work. However, our nasal spray products were inherited from someone else and I was not personally involved with the decision making process during development or drug application submission, so please keep that in mind.

1. The stoppers and glass vials that directly contact the spray fluid are checked for bioburden as each lot of components is received.
2. The mixing and filling operations are performed in Grade D cleanrooms but the final nasal spray assembly into the final applicator is not done in a cleanroom.

A3: Thanks for the information.
Even though you were not involved in the set up have you had experience of being questioned about the practice by regulatory bodies and explain the reasoning? how was it received etc..

Im assuming that in your product the fluid will also then flow through the applicator into the patient. do you do any testing of these applicator parts to show low bioburden and have assembly controls to then minimise any additional contamination risk?

i can see good points for both situations (in a clean room and not in a cleanroom) for assembling the device around the sealed vial/bottle. if you are testing for micro via the final the shot collections through the assembled device then you should pick up any challenge from the whole process. overall it is if we deem acceptable risk of this assembly part outside a cleanroom that could add contamination and result in a failure to meet spec.

i just cant see any clear instructions on this step of device assembly and hoping it is how we can justify the practice.

A4: Do you have any guidance documentation to reference against? i completely understand this thinking and am trying to determine what i have to do or can take a risk assessment approach and justify.

A5: Assuming the product will require medical device file 510K. The design history file must include a proper risk assessment of all decisions made from component testing, manufacturing, filling and assembly. The risk assessment should reflect the determination of risk factors, their failure mode effect analysis. Decision made based on such well laid out risk assessment and documentation will be accepted by regulatory agency. And if your device application requires approval, this will serve as very good evidence for your decisions.

extraneous gases in compressed air

Recently we've been asked to test the compressed air system for extraneous gases.

What gases do we have to test for and why?how do we perform the analyses?

Any references will be appreciated.

A1: The Compressed Air & Gas Institute (CAGI) defines those contaminants that need to be removed or reduced from industrial use compressed air. These contaminants fall into four categories:

- Particles
- Water
- Oil
- Microorganisms

ISO 8573-1:2010 is a compressed air quality specification that addresses these contaminants by providing a range of purity classes for particles, water and oil (No class is defined for gases or microorganisms).

ISO 8573 refers to gaseous contaminants as: carbon dioxide, carbon monoxide, hydrocarbons (C1 to 5), sulfur dioxide, nitric oxide, and nitrogen dioxide. If your product could be adversely affected by a particular gaseous contaminant, then it should be considered in your Risk Assessment and engineering controls, and monitoring specifications.

Would one perform USP <61> or <81> on process buffers with antibiotics & interpretation of <1227>?

if given an in-process sample of a buffer supplemented with antibiotics, would one perform USP <61> using neutralizers for the antibiotics?

OR- would one just perform USP <81> to ensure the effectiveness of the antibiotics in the buffer and go from there?

Also, in USP <1227> in the first section, the following is written: "When the product displays intrinsic antimicrobial activity for a given microorganism and, given this antimicrobial activity, the risk of microbial contamination is low, the method could be considered as fit for the purpose of providing a strong rationale."

How is the following part to be interpreted; "the method could be *considered as fit for the purpose of providing a strong rationale.*" ?

Validation of microbial testing methods

Where microbial testing of a drug product is carried out, the method should be validated to confirm that the product does not influence the recovery of microorganisms. When the validation is completed, do we need to apply also periodic validation (e.g. once a year, every two years) to confirm that the method is still valid?

A1: Reverification of validated microbial testing method is an evolving concept and there are several factors- was there any processing change, raw material substitution, new supplier of preservative, new API process, packaging component change, etc. that might affect the recovery? On the flip side, were there any new microbiologists performing testing, was there a change in media composition, etc.? Each of these by themselves might not have significant impact. However, there might be cumulative effect of two or more factors, which will impact recovery of a method that had marginally acceptable recovery to begin with. Having a strategy with pre-determined factors supporting the decision and following that schedule across the organization might be a way to go. So, during the inspection, it is clear that you considered it and you have a plan.

A2: I recommend that you view method suitability testing as a qualification not a validation as compendial methods being official are considered validated.

Method suitability testing would only be repeated if there was a significant change, e.g., change in formulation, manufacturing process or test method.

A3: To answer the question, once the validation is complete, a periodic review isn't necessary unless there is a modification in the product or method of analysis, then a validation needs to be carried out. To also add, if a new staff is also employed to the office or for the case of competency assessment, that can be carried out.

A4: No, there is no periodic validation for test methods. Additional validation is appropriate when you make changes to the test method or product. Changes to testing conditions can also drive validation activities.

A5: You'd certainly need to repeat method suitability testing if the formulation changes. Any process changes may need to have a risk assessment, to determine if these changes impact the formulation of stability characteristics (this would be performed as part of the process re-validation approach). Otherwise, there is no need to repeat method suitability.

You didn't describe what "microbial testing" types you're referring to (e.g., sterility, MLT, bioburden, etc.). That being said, you may have to perform partial method suitability testing for non-sterile products if you find an objectionable organism in (e.g.) a raw material, using that organism. You may have to perform partial method suitability testing for sterility if you have a sterility test or media fill failure, using that organism.

A6: No you are not required to repeat the method suitability testing with each staff change.

The expectation is that the analyst will have the education, skills and experience to follow the method as written in the SOP and successfully conduct the test.

Challenge testing on w/o emulsions

I work for a cosmetic company and I carry out challenge testing on "oil in water" emulsions. There is a need to perform challenge tests on "water in oil" products. I use a rich broth that contains polysorbate 80 at a concentration of 5%. Although the Method Suitability test shows that the concentration of polysorbate in the broth is suitable for testing these "water in oil" emulsions, I cannot see the product being dissolved in the broth. Is it possible to tell me how they could be dissolved?

A1: Try DMSO (dimethyl sulfoxide) with minor concentration of 1-2 % hope it works.

A2: Hi, you can also heat to not more than 45 degrees Celsius using a water bath, that would help.

A3: There is no need to use DMSO for an oil-in-water product emulsion. DMSO should only be used to dissolve anhydrous product samples that are not water soluble. An oil-in-water product emulsion is not a water insoluble product formulation. In addition to Polysorbate 80, I would include a 0.5% concentration of Soy Lecithin to dissolve an oil-in-water product emulsion in a plate count diluent because Soy Lecithin is an emulsifier.

To conduct challenge testing, it should be more than sufficient to vortex an aliquot of an oil-in-water emulsion to have a homogenous dispersion of the oil-in-water product emulsion if you are using Polysorbate 80 and Soy Lecithin as part of the plate count diluent.

A4: Heating a sample aliquot to a temperature to be no more than 45C will not break apart an oil-in-water product emulsion. It is normal to use temperatures higher than 45C to form oil-in-water product emulsions during compounding. Preservatives are usually added to an oil-in-water product formulation after post emulsification temperatures.

I do agree that sometimes heating certain raw ingredients with a poor water solubility to a temperature of 45C will aid in dissolving them in the water phase of a product formulation. Parabens are an example of a raw ingredient that can be added to the water phase of a product formulation by using heat.

A5: Sterile Isopropyl myristate is used as a non-toxic solvent for some substances. It's indicated in USP 71 for sterility testing as an option.

A6: Am sure you are aware In USP 61, the table showing sample dissolution/preparation speaks on heating as i mentioned on oily samples. I added that as he has mentioned the use of tween 80 already.

A7: I think that the question from Giolina is for w/o emulsions (water in oil, see the subject of the email), not for oil in water (o/w) ones, which are the object where some of your answers are being addressed to. The first are really difficult to dissolve in common diluents.

A8: An oil-in-water emulsion is not really oily. Oil product formulation mostly consist of mineral oil such as a bath oil. The usage of oil-in-water emulsions are very rare for drug product formulations. They are mostly found Instead to be cosmetic product formulations. I have found that most drug microbiologists have not and will never see an oil-in-water or a water-in-oil product emulsion in all probability because they are working with simple aqueous product formulations from an ingredient perspective with the exception of some creams and ointments.

The USP is very good in how to test aqueous product formulations, but the guidance in how to test what I call atypical product formulations Is somewhat lacking and not sufficient from my perspective. To perform testing on these atypical product formulations, it comes mostly from experience in performing microbial content and challenge testing on these types of product formulations and not by just reading a USP chapter. Part of this experience comes from knowing how to formulate product formulations in which most microbiologists have a lack of understanding and why certain ingredients besides preservatives are present in a product formulation.

Propagation of bacteriophage

I would like to know if it is a general practice to have a separated laboratory for propagating bacteriophage from laboratory which mainly manipulating bacterial culture, to minimize the risk of contaminating the reference strains of bacteria? Or it is acceptable to handle both phage and bacterial culture within the same laboratory?

A1: Everything depends on how strict your aseptic techniques are. In general, we do both in the same laboratory, but without proper training and cleaning regime you may have some problems. Many things depends on phage you are going to grow.

A2: Bacteriophages are highly specific for a bacterial strain so other bacteria handled in the lab will not be susceptible.

As bacteriophage contamination can be spread from the testing laboratory to the manufacturing facility contaminating the bacterial cultures used in your biopharmaceutical production.

What are you using the bacteriophage for in the lab? Is it for method suitability or as a positive control? Options are using containment like a biological safety cabinet or an isolator, using UV light to decontaminate work surfaces or moving the testing to a contract testing lab.

In-process Bioburden requirements

I'm trying to compile a list of requirements (either official or PDA publication) defining in-process Bioburden sampling / testing. Are they monograph specific per product (and / or water type used in the process per 1231), or are there regulations / standards out there that I'm glossing over?

A1: You should be aware that the USP does not specifically address in-process bioburden as USP <61> is provided as the microbial enumeration test when there is a microbiological requirement in the USP monograph.

A2: There is a helpful article written by the BioPhorum Operations Group (BPOG) entitled "Microbial Monitoring for Biological Drug Substance Manufacturing: An Industry Perspective" The corresponding author is David Bain - a BPOG facilitator [log in to unmask]

A3: In-process bioburden testing is considered as in-process test and BET testing is considered as in-process check , and these are considered as most important incase of biological products . Requirement of in-process testing will come from process expert team.

Media Fill temperature

We previously incubated media fills at 20-25C for 14 days. We intend to change to 20-25C for 7 days followed by 30-35C for 7 days. What level of justification is needed for this change? Is a risk assessment enough? Do we need to do a study with microorganisms ?

A1: Ironically it is more challenging to justify a change than justifying the original procedure.

My first exposure to media fills was with large volume parenterals and we incubated the media fill at controlled room temperature for 14 days in product storage and inspection area as we had no walk-in incubators, The incubation conditions were justified by industry practice and growth promotion testing.

The regs suggest any temperature between 20- 35 degree C that is justified. My opinion incubation at 28 degree C for 14 days may be optimal.

A2: I think you probably need to create your internal justification. Was this mandated by a regulator? If so, you should probably ask why. Was there an organism or set of organisms you can't recover?

Using two temperatures is very common so that's not an issue. You'd probably need to show you can recover something like Cutibacterium acnes (previously Propionibacterium acnes) in addition to the 5 classical USP organisms. That's just one example, you may choose other relevant environmental samples but I know C acnes is one of the more difficult to recover EM contaminants.

A3: You may be able to refer to the results of the PDA 2017 Aseptic Processing Survey (TR 45010) on media fill incubation parameters:

Single temp in the range of 20-35: 8% of respondents

Two temps 20-25 and 30-25 in sequence: 84% of respondents

Two temps 30-35 and 20-25 in sequence: 8% of respondents

A4: do not set the bar too high. TSB incubated aerobically is not that suitable for the recovery of C. acnes. Also the skin microbiota does not grow well at 20-25 degree C.

A5: Is there any documents referring your suggested practice?

A6: This is from the FDA Guidance for Industry Aseptic Processing:

- 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. Incubation temperature should be maintained within ±2.5°C of the target temperature.

- Incubation time should not be less than 14 days. If two temperatures are used for the incubation of the media filled units, the units should be incubated for at least 7 days at each temperature (starting with the lower temperature).

A6: What is the heading of that guidance? Is it journal/annexure/PDF?

A7: It's a PDF....FDA Guidance for Industry Aseptic Processing....search that phrase.

A8: The 2004 FDA Aseptic Processing Guidance 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 oC. Incubation temperature should be maintained within +2.5oC of the target temperature.

Incubation time should not be less than 14 days. If two temperatures are used for the incubation of the media filled units, the units should be incubated for at least 7 days at each temperature (starting with the lower temperature).

A9: I will admit that I have been out manufacturing sterile product formulations for awhile.

I fully understand the question about incubating media fill vials at 2 temperatures. Some things do not change in this aspect.

However, I have a question concerning this post about the test organisms used for growth promotion testing of media fills.

Why would anyone use Cutibacterium acnes as a growth promotion test organism for a media fill? Generally, this organism is an anaerobic microorganism that is present on the skin. I fully understand that it is possible to isolate this organism in sterility testing of a sterile product by using FTM, but it only grows in TSB that has been supplemented with defribinated sheep blood that is incubated under anaerobic conditions besides using Reinforced Clostridial Medium. I believe that TSB is mostly used as the microbial growth medium for conducting media fills. Is this still correct? I assume that it is. I can understand that it may be possible for Cutibacterium acnes to grow in sealed TSB glass ampules of a media fill. Not sure with sterile containers with a rubber stopper.

I have 2 questions for the group . Are people using Cutibacterium acnes as a growth promotion test organism for TSB media fills? Is it standard practice for positive media fill containers for microbial growth would be identified to the genus/species level? I suspect that this practice has not changed over the years. Has anyone ever isolated Cutibacterium acnes in a TSB media fill container?

A10: I believe, General practice for Growth Promotion is as below:

1. If Media fill is done under Anaerobic condition (Nitrogen blanketing), Clostridium sporogenes (CS) is included as one of the organism.
2. For Aerobic or An-aerobic media fill, in addition to routine organisms(SA, PA, EC, Salmonella, AB, CA,ML,BC and in-house isolates (GPR, GNR, Yeast & Mold) are also used.
3. NMT 14 days of incubation (Either 7 days at 20-25 C followed by 7 days at 30-35 C) or 14 days at 20-35 C is a general practice.
4. We do identify up to Species level as this is a rare occurrence and it provides additional information to identify the cause.
5. I have seen P.acne from Aseptic fill on Day-14 from FTM during sterility test.

A11: I always hesitate to say what P acnes can't do. I have seen appear in all manner of ways, from extremely air intolerant to very air tolerant and on TSA/TSB (unsupplemented). The same culture adapts quite quickly between states if it has just enough resource to survive long enough to do so.

I've also performed a study keeping it in an extremely low moisture dry powder environment. This documented that is was able to persist in a viable state for a number of years. If I were running an anaerobic media fill, and it is a known environmental of the facility I would include it as the in-house challenge microorganism.

A12: Salmonella used as a growth promotion organism for a media fill?
In the majority of circumstances as with environmental monitoring we find the use of a general microbiological medium like TSB adequate.

A13: I sort of broke my own internal rule....don't make things harder than they have to be! I always think of C. acnes as a great example of an in-house EM isolate because it's a known personnel isolate, relatively common, and it's a slow grower.....the worst-case scenario. It was an example of a bug that may be found in cleanrooms but probably not the best example of what organism to add to a growth promotion. Not that it can't be done but I frequently tell people not to choose difficult in-house isolates that might be inconsistent because I've seen that happen and customers can have a difficult time growing them. I have seen C. acnes grow in TSB and TSA without any supplements but it may not be the best EM isolate for growth promotion of media fill TSB.

A14: There is an excellent article, "Environmental and Personnel Monitoring Programs—A Risk-Based Case Study of Cutibacterium acnes", by Ed Tidswell and Kenneth Boone, published in the recent PDA Journal of Pharmaceutical Science and Technology: https://journal.pda.org/content/74/4/408.abstract?etoc

Microbiological quality requirement for drug substance (API)

As per the USP <1111> the microbiological quality requirement for the drug substances (API) is only TAMC & TYMC, there is no any requirement mention for specified microorganisms.

I have question, it is required to perform the test for specified microorganism for drug substance (API) which are use either in non-sterile formulation and sterile drug preparation?

I want to know across industry practice. Any ref. for specified microorganism for drug substance?

A1: This level of detail is not included in USP <1111> as the drug substance may be used in a sterile drug product or different non-sterile dosage forms. The recommendation may be viewed as a default

microbiological specification and the drug manufacturer based on a risk analysis would need to set an appropriate specification or refer to the specification in the USP monograph for the material.

A2: If your Drug substance is intended for sterile preparation you can perform TAMC&TYMC because final drug product is injected into patient as sterile where no microbes will be required.

If your Drug substance is intended for Non sterile preparation " specified organisms"should be tested because non sterile drug preparation is directly consumed by patient.

Regarding GPT testing of liquid broth media

I have a question regarding growth promotion test of liquid media .

Is there any guidelines which suggest to perform growth promotion test of liquid media in duplicate tubes?

A1: The USP <61> states the following:

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 <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>.

When using the test we use a single plate for membrane filtration test, duplicate plated for a pour plate test and three or five tubes for a MPN test. However, the section of the chapter does not specify the same number of plates or tubes as in the test so using one plate or tube for growth promotion testing would meet the requirements of the test.

A2: 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 occur. We have to confirm just growth observed or not. For liquid media not required to test in duplicate.

A3: One more thing I want to ask here, whenever it is written compared with previously tested and approved batch of medium, does it mean gpt should be run parallel with new batch of media and a older batch.

A4: That has always been my interpretation and practice, yes. I don't know of any other way to do it.

A5: You can compare the gpt test results of previous media with current test media.if you have been qualified your media shelf life.

EM qualification question

I have a question regarding facility EM qualification. During EM qualification, should we use Volumetric air sampling OR settling plate method for Non-Sterile facility. What is your opinion?

A1: You have use both methods for qualification for better assessment of area.

A2: Settle plates are generally frowned upon as inaccurate but it falls in the "something is better than nothing" category since not everyone has access to an air sampler.

A3: Typically non-sterile product manufacturing areas are not designed for or classified as to air cleanliness standards. If you use active air sampling what acceptance criterion will you use?

For a balanced discussion on the issue I would refer you to USP <1115> Bioburden Control of Non-sterile Drug Substances and Products.

A4: My opinion is to use a viable sampler. I realize non-sterile MFG has lower standards than parenterals but I think it's hard to "qualify" a room with semi-quantitative methods. I'm sure it's been done and I don't think anybody could say you absolutely can't do that. The sampler could be used for many years, justifying the purchase.

A5: My opinion is to use a viable sampler. I realize non-sterile MFG has lower standards than parenterals but I think it's hard to "qualify" a room with semi-quantitative methods. I'm sure it's been done and I don't think anybody could say you absolutely can't do that. The sampler could be used for many years, justifying the purchase.

Chapter <60>

Is anyone performing chapter <60> testing at a sterile injectable manufacturing company, at any stage of manufacturing? Is there any reason one may do so and if yes, on what would the test be performed?

A1: I see that no one had responded back to your posting about using USP Chapter 60 in a sterile manufacturing facility.

In the introduction of USP Chapter 60, it is stated as follows:

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.

In reality, there is no reason for using USP Chapter 60 because the chapter is for non-sterile aqueous and inhalation products based upon the introduction of the chapter. However, it is stated in the testing product section that it may be used for the testing of pharmaceutical waters. It would be interesting to see if regulatory authorities would require Water-for-Injection that is used for making sterile product formulations be tested for the presence of Burkholderia cepacia complex by using the methodology of this chapter by having this particular sentence in the chapter.

By having this sentence present in the chapter, I would suspect that you would be required to conduct Burkholderia cepacia complex of collected Water-for-Injection samples because it is kind of vague of what is meant as pharmaceutical water in the chapter. From my perspective, the presence of any Gram-negative bacterial species in Water-for-Injection samples would be objectionable.

A2: As WFI is not used for the manufacturing of non-sterile products there would be no reason to test it for Bcc.

A3: I do agree that WFI is not used in the manufacturing of non-sterile products. However, it is stated in the chapter that it is recommended pharmaceutical waters should be tested for Burkholderia cepacia complex.

Some people may think that they have to test WFI because it is a pharmaceutical water. The presence of the term "pharmaceutical water" is misleading in the chapter and people may think otherwise. It would have been better if the chapter had stated that water that is used in the manufacturing of non—sterile should be tested for the presence of Burkholderia cepacia complex to prevent people in applying this chapter to WFI.

A4: With so many industry experts being part of the USP committee, how come USP keeps issuing chapters with such confusing terms? Why no one holds them accountable for providing clarity? This always happens in microbiology sections and not in analytical.

A5: The chapter does not make that recommendation.

It states in the introduction the following:

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.

It does caution that if you apply the method as an optional test for pharmaceutical grade water that you use a 1:10 dilution. There is no recommendation that purified water be routinely tested using USP <60>.

A6: All new and revised USP chapters are published as in-process revisions and are subject stakeholder review and comment.

The comments, the USP responses and changes are published on the USP website so we are accountable.

A6: It’s because of Murphy’s law of Microbiology.

“Despite the best conditions of time, temperature and humidity, microorganisms will always do whatever they damn well please, especially when your job depends on it” (thanks [name redacted] for putting that in a presentation 15 some odd years ago).

Unfortunately, Microbiology is rarely “analytical” there are situations where any assay may be deemed critical and others where a risk assessment indicates that the same assay should be applied, optionally.

If you’re looking for a straight answer, you certainly won’t find it in Microbiology.

[name redacted] (a USP Microbiology guru himself) has already elaborated on [name redacted] comment.

Meanwhile, I’ll grab popcorn while waiting the responses!

A7: I think the operative words are "pharmaceutical" and "Optional". With USP chapters under 1000 I think this type of wording can cause confusion. I specifically asked the FDA about this topic at PDA Micro in 2019 and I was told it's a firm "no" on USP being intended for parenteral manufacturers as it's intended as a non-sterile product test. I know the intent was to give an additional method for testing water because that's where Bcc often resides and if firms want to test their water, this is one validated method. That being said, using the term "pharmaceutical waters" is very broad and is probably causing the most confusion in this chapter. After speaking with you (indirectly) and the FDA at the PDA, I walked away with the impression that USP 60 is only intended for non-sterile, aqueous product manufacturers. That being said, through this forum, we've discovered other non-sterile "non-aqueous" manufacturers who make dry powders for inhalation, who also test for Bcc because their patient population is at risk. I believe it is a full expectation that every non-sterile, aqueous manufacturer should be testing for Bcc but that other firms will also perform the testing because their patient population is at risk, even though the product is non-aqueous.

If a firm is producing a non-sterile, aqueous product and are performing USP 60 on the final product, that will fulfill the testing requirements and if Bcc has been found in the water system in the past, I would think testing the water system more frequently to prevent product contamination would be a good idea.....that's just my 2c.

A8: You hit the nail on the head. What is the interpretation of pharmaceutical grade water? Is it WFI or USP Purified Water? The are both used in the formulation of pharmaceutical products. The term of pharmaceutical grade water is too vague and someone may take it that WFI needs to be tested which was not the intent I believe of the chapter.

I hope in the next revision of the chapter that there is more clarity of what is pharmaceutical grade water that needs to be tested for Burkholderia cepacia complex.

A9: The debate back and forth is an evidence that there are ambiguity when it comes to microbiology sections in USP- an area that certainly could use some improvement. We are all accountable to provide comments but not all comments are addressed and USP chapters continue to be issued with ambiguity.

A10: The purpose of official USP test methods is to provide a test, if a requirement for the absence of Bcc is added to a USP product monograph. As stated in the chapter, the test is directed towards aqueous, non-sterile dosage forms.

Given the ingredients, manufacturing process, and product microbiological and physicochemical attributes of a compressed tablet or powder-filled capsule, in my expert opinion, I would not recommend screening solid, oral dosage forms for the absence of Bcc.

The expert committee was aware that companies may use the test for water monitoring and added the caution suggesting dilution of the enrichment broth may be necessary to recover Bcc. No endorsement of this monitoring should be implied.

A11: I can't speak for all regulatory authorities but the FDA almost never requires speciation of WFI isolates due to the significant downstream sterilization controls that are implemented to neutralize the bulk drug product bioburden. Speciation could be requested to investigate a catastrophic sterility assurance failure but it's rare. The choice not to speciate WFI organisms is supported by a risk assessment showing that the levels of microbial contamination in the raw materials (including water) and pre-filtration bioburden cannot withstand the sterilization process and are not of a sufficient population to affect the purity, strength, potency or quality of the finished drug product. The same risk assessment process can be used to determine if testing for B. cepacia complex in pharmaceutical water systems used for the manufacture of non-sterile drug products is necessary. Usually it is not, but there are instances where testing water systems for B. cepacia complex may be beneficial due to factors such as manufacturing/product history, weak downstream controls, or the unique nature/intended patient population of the drug product.

A12: I'm in agreement with your statement that tablets should not be tested for the presence of Burkholderia cepacia complex.

For most aqueous non-sterile product formulations that have the presence of Burkholderia cepacia complex, the water that is used to manufacture the product is the likely source of the contamination.

In your posting, you had stated that the expert committee was aware that companies may use the test for water monitoring and added the caution suggesting dilution of the enrichment broth may be necessary to recover Bcc. No endorsement of this monitoring should be implied.

I would have not included the statement in the chapter as an option and just leave it for non-sterile aqueous and inhalation product formulations. If the USP expert committee has a concern about Burkholderia cepacia complex in the water that is used for aqueous non-sterile product formulations it would have been more appropriate for the USP expert committee to develop an actual microbial test chapter for purified water instead that is not a guidance chapter. Most of the microbial test methods that are used for the microbial analysis of water comes from Standard Methods for the Examination of Water and Wastewater. In this book, there is no enrichment test method for detecting the presence of Burkholderia cepacia complex in water samples.

Before this chapter had been issued, I would think that it was not a common practice to conduct enrichment testing on purified water samples. If identification is done, it is performed on microbial colonies

that are present on the pour or membrane filtration recovery plate. Furthermore, I would tend to believe that most people did not test their purified water samples for the presence of Burkholderia cepacia complex by conducting identification on recovered isolates unless the microbial counts were above the alert and action levels for a water system.

If the USP expert committee has a concern about the presence of Burkholderia cepacia complex in purified water system for non-sterile aqueous products which is justified from my perspective, make it a requirement that speciation be conducted on representative colonies from recovery plates in a purified water microbial testing chapter instead of placing this vague statement in the chapter for detecting the presence of Burkholderia cepacia complex in aqueous finished products that it is optional for pharmaceutical grade water. In addition, this purified water microbial testing chapter should not be a guidance chapter because the information in it is not requirement. If such a requirement was put in place for identification of all recovered colonies on all purified water samples no matter what was the count, it might cut down the number of product recalls due to Burkholderia cepacia complex contamination.

I also wonder whether Burkholderia multivorans and Burkholderia cenocepacia would actually be detected in a purified water sample by conducting enrichment. In a purified water system, the microbial population is not a monoculture environment. Several different species can be present within a purified water system at the same time due to the microbial variability of the source water. In addition, water borne organisms that have a faster growth rate may inhibit the detection of Burkholderia cenocepacia and Burkholderia multivorans by using TSB enrichment of USP Chapter 60 due to their slower growth rates.

In regards to my statements on this subject, I believe that some people will agree with me and others would like to burn me at the stake by providing the wood because I question the expertise of the USP Microbiology Committee. However, I believe that the purpose of this forum is educational by having the free exchange of different viewpoints on a subject. This is what makes Microbiology fun. We all learn in the end.

A13: [name redacted] you and other members of the PMF should be aware that the USP Microbiology Expert Committee is not responsible for water chapters and monographs.

A14: Water is an important ingredient in non-sterile product formulations and for cleaning and sanitization of manufacturing equipment.

Why couldn't there be a microbial test method chapter for water be written like Chapter 60? This should be a joint effort between the USP Microbiology Committee and the committee that is responsible for water to establish a test method chapter so that the confusion in how to test water samples no longer exists.

If the USP Microbiology Committee is not responsible for the water chapters and monographs, why make a reference in using USP Chapter 60 as an option for use in detecting the presence of Burkholderia cepacia complex in pharmaceutical grade water? By making this reference for testing water for Burkholderia cepacia complex, it looks like the USP Microbiology Committee had stepped upon the jurisdiction of a different committee.

Failed Neutralization in Suitability Testing

All - does anyone know what the USP recommends if the suitability test fails using neutralizing agents? What is the next step, can we proceed with the PET/AET on the product?

A1: What is the article failing to be neutralized and what have you tried so far?

A2: That depends. You can use neutralizers in a membrane rinse fluid and in the final medium. You can also dilute the preparation under test as long as the dilution is not too high that you cannot make a determination if the required log reductions are achieve that the relevant time points. IN fact, USP 51 states if you need to make significant dilutions you may have to inoculate with a log higher concentration of challenge organisms.

Or, you can do a combination of dilution and neutralizers.

The expectation is that you employ all of these strategies.

A3: I believe the USP indicates that a repeat with different neutralizers, larger solution of product to diluent, etc are all acceptable next steps. Until you have validated that you can neutralize the product, you can't move forward with PET.

A4: I have a question on your post. Did you failed neutralization against all of the 5 challenge test organisms? If the product had failed against all 5 challenge test organisms for the AET, the product may be hostile to contaminating organisms and challenge testing might not be required. See ISO 29621 for the factors that makes a formulation unsusceptible or hostile to microbial contamination.

If one organism has failed neutralization, I would recommend that challenge testing still be conducted with the organism to show that it is not possible for it to survive or proliferate in the formulation. An example of a product that has neutralization issues is Zinc pyrithione antidandruff product formulations in which it is very difficult to recover Candida albicans for an AET. It is not surprising because dandruff is caused by a yeast and Zinc pyrithione is active against the yeast (Malassezia)) that causes the condition.

A5: Chapters 51/61 and 62 of the USP specify 3 methods, these methods can be combined in order to obtain the neutralization, if in the end nothing works you can evaluate and apply the resolution of these chapters in which you can specify that the product is little or impossible to become contaminated. but you should try to exhaust the possible neutralizations.

A6: Chapter 51 is very specific in the methods to be used as well as increasing the chances of success. I also recommend reading the additional supplement of Chapter 51 if you need it. Additionally the method you use to neutralize will be the daily analysis so the final method.

A7: A large number of products can be resistant to neutralization, one of many cases may be anti-inflammatory, antipyretic, and analgesic activity, these products in formulation enhance preservatives, making neutralization and recovery more difficult, one tip is that you should study the formula of your product and on the basis that it make decisions.

strain typing for investigations

Does anyone have experience of using strain typing methods to support investigations (biofilms/in-process bioburden coming through from Upstream and persisting post inactivation stages etc.)? Any published articles? Any input on the awareness within regulatory circles in the use of such methods and how it might be interpreted by an auditor will also be appreciated.

I'm thinking MLST to show that a specific type of a certain organism that seems to break through in post-inactivated stages are linked to organisms seen in-process at pre-inactivated stages but at a lower number. Review of cleaning/sanitisation procedures etc. are ongoing. I am also trying to show that this specific phenotypic variant is more resistant to inactivation steps/can form biofilms - is there value in this in front of an auditor where it could muddy the water if no causal links are actually found! Any opinions/ experience would be appreciated.

A1: I did co-author a publication that used strain typing to show the equivalency of QC organisms.

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

Loss of Power in Aseptic Pharma. Plant

Does anyone have a time limit for loss of power (HVAC), after which you would do a 3X clean? I was thinking 15 minutes, but some people thought 30 minutes would be acceptable. Are there any guidance documents for this issue?

A1: Even a momentary loss of power could have an effect on air handling systems and other utilities that are not backed up properly by alternate power, that could adversely effect environmental conditions in your suite.

A2: Sometimes the important point to focus upon is the "Loss of Class and Positive Differential Air Pressure" rather than only the loss of power. Some HVAC supply systems "wind down" slower than other. That gives operators time to contain and leave the area. Likewise, there is also a delay in the loss of Class 100 and 10,000 (old school) classification . These may give you a 30 second delay to contain the area.

It is equally important to know how long it takes to return to the proper classification and differential air pressure after a power loss interruption. Most HVAC systems or SCADA systems can tell you the duration of the "power loss" interval. This timing fits into the control strategy that you can develop with understanding the other items I mentioned above.

A3: This should be based on environmental recovery documentation; best defense is actual incident monitoring (Viables/Non Viables) correlated to actual CFM supply/exhaust monitoring documentation during the events. It may be quite possible to defend a 15 minute/ 30 minute safe period since most HVAC systems have flow lags within these short time periods where the actual flows are able to maintain control. Again should be documented/validated. If you have historical cfm monitoring data a retrospective review of incidents correlated with a validated historical environmental monitoring program may give you a defensible position.

A4: Years ago, during the era of "Brown-outs" in California, I performed viable and not viable air evaluations in an ISO5/7 cleanroom before, during and after a power outage. We placed sampling devices on the floor next to air returns and also at work heights on filling equipment in the middle of the room. As expected, we saw non-viable counts increase at locations in front of the air returns when the power was turned off . However, surprisingly we saw more viable counts at the filling line than near the air returns. That phenomenon occurs because the (less-clean) air inside the air returns up to the air handler collapses during a power outage, pushes out through the air returns and flows off the floor and toward the center of the room. So if you have any power outage, you run the risk of contaminating your ISO 5 area almost immediately.

If you want to have a 5-minute rule (like when you drop a piece of candy on the floor), you need to generate some data (airflow study and similar viable & non-viable air sampling that we performed), and write a conclusive risk assessment to defend the practice.

Best advice: Wash your hands, stand 3-6 feet away from another gowned operator, and wear a sterile mask.

By the way, don't pick up the candy and eat it in the cleanroom!

A5: Facilities should establish a time limit that AHU's/HVAC's shutdowns, along with pressure differential excursions, are allowed without requiring an assessment of product impact, a triple or major clean, and conformational environmental monitoring for release of the area. The decision to release a room without action should include conditions such as minimal or no traffic in the area, if doors need to remain shut, no open product, and a time limit. Most facilities should be able to withstand an AHU shutdown of 1-2 hours without having to clean and monitor as long as no personnel were in the area during the event and the environment was not breached. The duration and activities allowed would have to be supported by an environmental study. This scenario as well as other events impacting critical environments is discussed at length in our book chapter " How Issues Related to Utilities, Surfaces, and Practices Impact Cleanroom Environments" by Jim Polarine and Beth Kroeger. Source: Madsen, R.E, & Moldenhauer J. (2016).

Contamination control in healthcare product manufacturing, Volume 4. River Grove, IL: Davis Healthcare International Publishing, LLC.

Pressurized product GP

We have a product that is being tested for "final product sterility" so the product is incubated for 7 days then tested for contamination. The issue is that the product develops a positive pressure inside its container so can create an aerosol as it depressurizes on opening. We are looking to perform growth promotion testing with the standard USP organisms so we anticipate A brasiliensis will have developed spores that may be released into the BSC on opening the container.

Our assumption is that the release of spores into the BSC is not desirable so we are looking for ways to address the issue and perform the test. Has anyone come across this issue and found a way around it?

Online TOC meter

Can anyone guide on using online TOC meters in purified water systems, especially during installation. When offline systems were used from years, definitely online systems are an advantage. Can it is installed in a system just after UV system after generation in purified water, or it should be at return loop.

What should be the PQ phenomenon and what should be the qualifications criteria for and online system. Does it happen that A line meter shows high conductivity and a low TOC. Both should be different meter at different places or both should be measured by one unit. Can conductivity be correlated with TOC.

Maintenance strains cultures for cryogenics

I would like to know how maintain bacteria and fungi cultures by cryogenics techniques (freezer -80°C).

My project is to reduce the amount of strain purchases per year, and I have many doubts about this.

If my original strain has 1 year of expiration, can I use it after that period using the -80°C maintenance technique? Or just in that period of validity? Because I read that I can use for about 5 years or more (USP 43 - chapter: 1117 - below).

"MAINTENANCE OF MICROBIOLOGICAL CULTURES
The original sample from the national culture collection or a qualified secondary supplier is resuscitated and grown in an appropriate medium. Aliquots of this stock culture (the first transfer or passage) are suspended in a cryoprotective medium, transferred to vials, and frozen at -30° or below, until use. If stored at -70°, or in lyophilized form, strains may be kept indefinitely. These frozen stocks can then be used to inoculate monthly or weekly working cultures. Once opened, do not refreeze unused cell suspensions after culturing a working suspension. The unused portion should be discarded to minimize the risk of loss of viability and contamination of the stock."

What materials I need to have to maintain this pharmacopeial strains?

A1: Use sterilized solution of 30% glycerol:70% nutrient broth to suspend the cultures, then freeze at -20deg C and transfer to -80deg C the following day or after 12 hours at -20deg C. That will give optimal preservation for all but obligate anaerobes. Aerobic and facultative anaerobes will like that just fine. We see stable recoveries of all our cultures and we have 36 months of data.

A2: I currently use a strain from Microbiologics (Kwik Stik and Accu Shot). Can I use this strain for cryogenics? I don't know how to start. ☹ If my original strain is passage from reference 2 or 3, can I use?

Rooms and grade zones in which microbiology operations could be carried out

I'm looking guidelines or recommendations that details zones or grade zones in which microbiology operations could be carried out.

The last one that i saw was Annex 2 WHO Good practices in microbiology labs where explain the follow:

Zone Installation	Grade	Proposed
Sample receipt	Unclassified	Unclassified
Media preparation	Unclassified	Unclassified
Incubator	Unclassified	Unclassified
Enumeration	Unclassified	Unclassified
Decontamination	Unclassified	Unclassified

For Suitability test or GPT where we used live microorganisms, we need to have a different room or we can used the same that we use for non sterile test? Which guidelines talk about this?

A1: I recommend as additional text the following ISO 17025 Paragraph 5.3 and ISO 7218 paragraph 34.1 premise 4.1.1.

You must complete the information according to the type of industry in which you work and have space with specifications equal to or better than where the product is elaborates.

A2: You can some Information in USP <1117> related to this question .

Using Stomacher for Mixing Extend release tablets (Metformin XR tablets)

Kindly advise , Is it suitable to use Stomachers to suspend Extend releasers Tablets (10mg of XR tablets in 100mg Buffered sodium chloride solution) to be used for Microbial Limit test for Oral solid dosage forms as per USP <61>?

A1: We use Stomachers to dissolve gelatin capsules or raw material which are harder to dissolve or mix. Please note, we cover this technique also as part of our individual microbial method validation for each product type.

A2: Yes I am a broken record but a risk assessment should determine that routine microbial testing of this solid dosage form is not justified.

To answer the question, stomacher that are more widely used in the food industry to test product can be successfully employed for pharmaceutical testing.

MLT method validation

How many batches is required to perform MLT method validation of non sterile products when harmonised pharmacopoeial method(USP 60,61 and 62)is being used. Please mention the guidance reference along with your comment.

Disinfectant for Bacillus spores

I have a query regarding disinfectant validation. How much effective is ethanol or 70 percent IPA against Bacillus subtilis? As per my results i am not even getting a single log reduction.

If alcohol is only inactivating the spores than what disinfectant is actually killing them? Currently i am using 70 percent IPA for cleaning of LAF , is there some other disinfectant recommend for the same.

A1: 70% IPA is completely ineffective against Bacillus. You'll need to use a sporicide. There are many on the market.

A2: Alcohols, like IPA and EtOH, will have virtually no effect on Bacillus spp. once they have developed their endospore. You should consider using one of the following:

* Hydrogen peroxide/peracetic acid compound
* Sodium Hypochlorite (bleach) at 0.52%; 1:10 dilution from "stock bleach" (5.25%)
* Hydrogen peroxide at 6%

Any of these should be tested against your EM isolate(s) for your SOP designated contact time and assessed for desired log reduction. You may also want to consider a rinse step (with either IPA or WFI) after contact time is achieved to reduce residues in your hood. Hydrogen Peroxide would be of lesser concern for rinsing due to its low, non-corrosive residues.

A3: Use Sporicidal to kill Bacillus.

A4: Alcohols are not effective for spores.

A5: The alcohols you mention are effective against vegetative cells of bacillus, but not spore forms. If you are testing with spores, I wouldn't expect any reduction.

A6: A solution of 60-70% Ethanol or IPA is not sporicidal at all. Alcohols will not kill spores. A high concentration of alcohol can prevent germination or the formation of spores, but they will not kill them. To kill spores, you need to use a disinfectant that is sporicidal that contains an active ingredient such as glutaraldehyde or peracetic acid.

A7: Bacillus spores are notoriously resistant to a variety of common disinfectants. For surface cleaning only harsh agents like SporKlenz or Bleach are appropriate. This also depends on the contact time you allow. You will find that other disinfectants such as Vesphene or LpH do nothing to your spores.

The level of resistance may vary depending on the exact strain you use, sporulation conditions, method of application etc. Alcohol and other drying agents are good when the bacteria can't face low water activity. It will be surprising if you actually detect any reduction that you can call significant.

A8: While 70% IPA demonstrates excellent bactericidal activity, alcohol lacks activity against well-formed spores. It is essential to include a sporicide such as hydrogen peroxide/peracetic acid blends, VHP, or sodium hypochlorite to control bacterial spores in cleanrooms.

A9: As many have said alcohols are ineffective against Bacillus spores.

Sporicides like peracetic acid, sodium hypochlorite, chlorine dioxide at the correct in use strength and wet contact time will do the job. They work work quickly.

Hydrogen peroxide and glutaralhehyde are sporicidal but are slow acting.

Sporicides are generally oxidising agents so take care as they are corrosive on many materials like metals even low grade stainless steel They require care when handling so correct ppe is vital too.

A10: Commonly I was using the stronger aromatic alcohol Phenol. But with great care, not to inhale

A11: Based upon the responses that you are receiving on your posting, I see that people are recommending the usage of chlorine to kill spores. To a use chlorine solution as a disinfectant, it is

generated by using sodium or calcium hypochlorite. I will admit that a that a chlorine solution has excellent activity against spores. However, you should be aware that there are some downsides in using chlorine as a disinfectant. For example, it is well known that chlorine solutions are sensitive to light exposure and temperature that can decrease their antimicrobial activity. Because of this, it should be prepared freshly. In addition, the antimicrobial activity of a chlorine solution is inactivated by organic residues that may be present on equipment surfaces and may react with metal surfaces by pitting stainless steel surfaces such as 316L. If an equipment surface is pitted by using chlorine, it provides an area in which microorganisms are able to hide from a disinfectant. When a chlorine solution is being used on equipment, please be aware that you may be taking care of one issue, but could be causing another.

A12: A listing of sporicidal disinfectants can be found on the EPA website https://www.epa.gov/pesticide-registration/list-k-epas-registered-antimicrobial-products-effective-against-clostridium along with potential sterilant compounds, if desired https://www.epa.gov/pesticide-registration/list-antimicrobial-products-registered-epa-sterilizers. As with any compound, one should verify material compatibility and verify the required PPE.

Raw material sampling area

A nuclear pharmacy wants to set up a raw material sampling area. Raw material receipt and sampling is an occasional occurrence. There are two options:

1 Sample in the sterile testing room(ClassB).
2 Sample in the nuclear pharmacy.

The QA is afraid of contaminating the pharmacy but the QC is afraid of contaminating the sterile testing room. Which choice is best?

A1: Have a sampling booth installed in the receiving area. The classification should be the same as where the materials are handled in the compounding area.

A2: Is there a particular regulation and/or guidance document that states the raw material sampling area should be the same classification as the compounding/manufacturing area? I’ve searched but perhaps have missed it. Currently our RM sampling area is ISO 6 and the manufacturing areas are ISO 7 (set up by previous management) and I am trying to justify change the sampling area t ISO 7. It would be great if I had a particular document to reference.

A3: I reviewed the 21 CFR Part 211 GMP regulations for specific requirements and see none. However, sampling of drug substances and excipients should be conducted in sampling booths with the same air cleanliness standards or better as when the drug product is manufactured.

I recall working for a company who received a 483 citation for this deficiency.

Extension of Incubation Times USP 61, USP 62

If validated can incubation times be extended though weekends and holidays? If so, How can it be explained to auditors about the change in incubation times? Due to the small size of our lab we cannot commit to weekend hours but sadly the lab is behind on releases.

A1: USP <1117> Best Microbiological Practices states:

MICROBIOLOGICAL MEDIA INCUBATION TIMES Incubation times for microbiological tests of less than 3 days' duration should be expressed in hours: e.g., “Incubate at 30° to 35° for 18 to 72 hours”. Tests longer than 72 hours' duration should be expressed in days: e.g., “Incubate at 30° to 35° for 3 to 5 days”. For incubation times expressed in hours, incubate for the minimum specified time, and exercise good

microbiological judgment when exceeding the incubation time. For incubation times expressed in days, incubations started in the morning or afternoon should generally be concluded at that same time of day.

A2: In reference to your posting, you had asked a particularly important question that I feel most people would not comment on due to being afraid that a regulatory authority would see their response. It is not an uncommon occurrence today for most microbiology laboratories in not having staff members being present on the weekends and on holidays due to cost containment initiatives. To overcome this particular problem, I would like to suggest the following steps below to improve the operational flow within your laboratory:

· When trying to validate the microbial test methods in the USP Chapters of 60, 61 and 62 for a test sample, I always recommend that you try at first to validate the shortest incubation times for the plate count agars (e.g. TSA for bacterial incubation of 3 to 5 days and SDA/PDA for fungi incubation of 5 to 7 days), enrichment media and selective/differential agars. If you validate the detection time for each USP indicator test organism by using the shortest incubation time period allowed for a test sample that is present in each of the above USP test chapters, it is not going to delay the detection of an organism if a longer incubation time period is used for the plate count agars and TSB enrichment media to cover the weekends and holidays. I know what some people will say something about incubating plate count agar plates longer than indicated could cause issues with the possible presence of microbial spreaders. If you know from past microbial test history for a test sample that microbial spreaders are present, you conduct an agar overlay of the plate count petri dish by using 3 to 5 milliliters of melted agar to prevent spreaders in order to obtain a count if the plate count agar plates are incubated longer that the shortest incubation time period. In enrichment test samples using TSB and MacConkey Broth, the fastest growing organisms that is present in test sample will instead be detected because they will inhibit the presence of slow growing organisms and this fast growing organism can be subculture from the enrichment broths of TSB and MacConkey Broth no matter what is the length of the incubation period because you are growing the organism in an enrichment media to a concentration of greater than 10e6 CFU/ml.

· For periods that are not around holidays to cover weekends, I would conduct USP testing (60, 61, and 62) on a test sample on Mondays, Tuesdays, Wednesdays, and Fridays due to the fact USP Chapter 60 requires a minimal incubation time of 48 hours and USP Chapter 62 requires a 24 to 48 hour incubation period for E. coli in MacConkey Broth. If a 72-hour incubation period is required based upon the validation data for detecting the presence of Burkholderia cepacia complex strains in USP Chapter 60 for a test sample, I would not conduct enrichment testing on that sample either on a Wednesday or Thursday. By incubating a test sample in enrichment broth longer than 24 to 72 hours in TSB and MacConkey Broth, it is not going to change a thing for detecting the presence for each of the USP indicator organisms that are listed in USP Chapters 60 and 62. However, I would not recommend incubating the selective/differential agars in USP Chapter 60 and 62 to no longer than what is indicated in each of these chapters which is my personal opinion.

To prevent problems during an inspection of the microbial test methods that are used, I would suggest that it state that the method has a minimal incubation time that directly corresponds to the shortest validated incubation time period.

A3: We take this one step further and during method suitability we record the results at the shortest and then the longest times we allow (same plates). That way with each organism/sample we can be sure we are not going to see a difference that makes the method not valid. All our products are non-sterile and for oral/dietary use. As far as enrichment, it’s a little trickier. Some of our products do have bactericidal properties, so longer enrichment can be an issue. We even use an enrichment, then refrigeration prior to secondary selective enrichment for selective organism recovery. I agree with no for selective agars since each organism will react differently. Again – prove any variation from the method is valid.

Note: We have a long history with all our vendors and our microbial load is very low, so overgrowth or spreaders are rare.

A4: Using the shortest and the longest incubation time using laboratory culture during method suitability testing is flawed as the growth pattern of a pure culture will be larger colonies and not more colonies with longer incubation time.

With samples containing a mixed culture you are more likely to see both spreading colonies and an increased count when you extend the incubation time. The limits are related to the incubation time. An additional risk is a product may extend the limit if you extend the incubation time.

I like [name redacted] suggestion in scheduling your testing so you can comply with the incubation requirements.

Microbial Contamination of Product Packaging

Today, there was an interesting US product recall due to the presence of mold growth causing a breach in the outer package integrity for a swab product containing 2% Chlorhexidine gluconate in 70% Isopropyl Alcohol in which the product had been stored for greater than 6 months at temperatures of 86F and 75% relative humidity. The mold was identified as Aspergillus penicilloides. I do not think that the impregnated solution of the swab was contaminated with mold because Chlorhexidine gluconate has antifungal activity. From my perspective, this product would be considered as self-sterilizing due to the presence of both Chlorhexidine gluconate and 70% IPA and there would be no need to conduct microbial content testing.

How did the outer product package become contaminated with mold? It is my opinion that by storing a product under 75% relative humidity would not cause the outer packaging to absorb water unless the product had been exposed to a water leak. Because I had a similar experience in having mold growth occurring on the applicators for an unused click pen containing different aqueous product formulations that had been stored under high temperatures and relative humidity, I'm speculating that the root cause for the Aspergillus growth in this product recall was due to the presence of a sufficient amount of headspace in the package that allowed for the evaporation of water from the 70% IPA formulation component. I have seen this occur before in jar tops that had mold contamination for certain products that had been stored in warehouses that were not air conditioned due to the presence of a large amount of headspace in the packaging. In all probability, it is very likely that the evaporated water from the impregnated swab had condensed onto the outer packaging layers causing the paper layer to become wet which allowed mold growth to occur on the outside of the package to cause a breach in the package integrity.

As microbiologists, we are mostly concerned whether the product is compliant with the QC microbial test specifications and whether it is adequately preserved. In general, we never look at the product packaging to determine whether it could lead to microbial contamination because we are just in our microbiology silos conducting testing and not looking at the big picture.

A1: I have a different perspective than [name redacted]. The backing of the outer packaging especially if it is cellulosic will adsorb moisture and support fungal growth As at 0.75, that may grow through and contaminate the applicator. I have seen this with other medical devices.

A2: Bar soap wrappers, esp. for high water bars shrink wrapped in bundles, often include preservatives. Paper brings its own fungal flora (spores),and susceptibility follows Aw dynamics as water is wicked and and evaporated water is condensed. Inventory control and FIFO van mitigate risk.

Variability among bacteria plating samples

I have been trying to determine if there is a standard for the amount of variation in plate counts within a group. For example, if I have a group with 5 individual samples and I calculate the CFU for each of the 5 samples, what kind of spread or variation is acceptable among the 5 samples? Suppose instead that I plate 5 aliquots from the same sample? What would make you comfortable with my results as a reviewer? The only info I have been able to find is from the original Breed & Dotterrer paper where they threw out samples that deviated more than 20% from the average. Is that the gold standard?

A1: For a traditional plate count method, a coefficient of variation of less than 35% for a microbial number higher than 10 CFU is generally expected.

A2: I would tend to think that the variability would depend on the actual number of colonies on the plates. USP <1227> has this wonderful table 3 showing the Error as a Percentage of Mean for Plate Counts and it is eye-opening. There are other papers documenting this as well with Scott Sutton's paper highlighting the best:

http://www.microbiologynetwork.com/counting-colonies.asp

For our ISO 17025 accreditation we perform proficiency testing between analysts with an unknown that has a sufficient count to avoid these massive error percentages. We typically see not more than 0.1 log10 difference in counts between analysts. I know other labs use other metrics as well but this process seems to have kept our ISO auditors happy.

A3: The Bioball was developed originally for the purpose of calibration. As such the counts are very accurate. If you are going to assess variability, you may find it useful to star with a count likely to be the same across plates. The late Dr Korczynski (Abbott) and I (Baxter) collected control charts across individuals and found quite a bit of variability in the counts, depending upon the person doing the counting. Use of the bioball, aided us in determining the actual count variability vs. the inoculation variability.

You might also want to look at the PDA bioburden recovery technical report. It is an old report based upon data generated at Baxter, working with a statistician and we found approximately 30% variation in counts. (It was based upon a Poisson Distribution but we rounded the variability to 30%.

A4: Issues around data integrity and the equivalency of automated plate counts to analyst counts has forced us to revisit the accuracy and precision of plate counts.

Typically we discount the limitations of traditional plate count methods. The precision of a plate count improves as the count increases and in the countable range of 20 to 200 CFU per plate the precision may range from 47 to 14%. Based on data I published I assigned a precision around 30-35% for a plate count.

The agreement between analyst counting the same plates is a different issue. I found a report on the efficiency of colony counting amongst 6 analysts with colony count range/plate of 20-200 with 339 plates counted/analyst was 60% of count within 5% of a photo count and 85% within 10% of the photo count. This indicated that a second person count to verify a count is problematic.

References:

Marino, G., C Maier and A. M. Cundell. A Comparison of the MicroCountä Digital System to Plate Count and Membrane Filtration Methods for the Enumeration of Microorganisms in Water for Pharmaceutical Purposes. *PDA J. Pharm. Sci. & Technol. 54(3) 2000 *

Jarvis, B 1989 Statistical Aspects of microbiological analysis of Foods Chapter 7 Errors associated with colony count procedures p95-116 Elsevier.

Vaccinate the world

If a Covid vaccine were available tomorrow for manufacture, how would the Pharma Industry produce 8 billion doses to vaccinate everyone?

A1: First of all, I would imagine that you don't need to vaccinate everyone. Similar to the Polio Eradication Strategy, a Targeted Mop-Up Campaign would be the idea method. There is no need to rush to vaccinate everyone in the countries that have little to no spread of the disease, or even every village/town in affected

countries. Simply vaccinating in the loci of outbreaks (the 'hot spots') would be sufficient. At the very least I would think this would knock an "easy" 5-billion off your projection.

Then you vaccinate the most vulnerable (i.e. people over age 45). The last statistic I read said that approximately 1 billion of the world population is over age 60. Once accounting for those that live in areas with no outbreak, I am confident you would have a very achievable number of people to vaccinate first.

We have a virus that - and I'm speaking optimistically here, although I know that you could put a negative spin on this - is only present on a host for 30 days, and only survives on surfaces for hours or days. Hot spots can be purged of the virus in a month - look how quickly some countries were able to control the situation with quarantine, contact tracing, and testing.

Guidance/Regulations for pipes to drains

Does anyone know a reference for the amount of slope of a pipe on the bottom of a tank to make sterile products to the drain? How should the slope be measured?

Also, does this same reference mention the 2 inch air gap from the end of the pipe to the top of the drain?

A1: I found this reference (pg 23):

https://www.chemsealinc.net/content/FreeGuide.pdf

Another reference:

https://www.arcmachines.com/industries-served/pharmaceutical-biotechnology-medical/asme-bpe-1997-standard

A2: I found it quite interesting that the author of the ASME piece (Dr. Barbara Henon) received her doctorate in biological sciences, did postdoctoral studies in neurophysiology and now writes articles on customers' applications of orbital welding. She has 12 years of field experience training orbital welding operators.

You never know where you're career path will take you I guess. Quite impressive.

A3: Most of the hygienic design principles for pharmaceutical manufacturing equipment comes directly from the dairy and food industry. In reference to your question about the size or height of the air gap, see Section 801.2 Air Gap or Air Break Required of the 2018 Uniform Plumbing Code.

The height of the air gap for indirect discharge between an indirect waste pipe (e.g. equipment drain) and the receptacle of the drainage system must be a minimum of 2 times the diameter of the indirect waste pipe if the size is over 0.5 inch. If the diameter of the indirect waste pipe is an inch or 2.54 centimeters, the height of the air gap needs to be 2 inches or 5.08 centimeters. You will see this air gap height requirement in a lot of state plumbing codes for food establishments that are located in the United States.

Most people think that only a 2 inch air gap height is just required between the equipment drain and the drainage system, but it is not because the height of the air gap between the equipment and drainage system is solely dependent on the size of the equipment drainage pipe.

endotoxin common practise

It is now "common practise" to test all raw materials for bioburden. Is it also common practice to test all raw materials for endotoxin?

A1: The short answer is no.

A risk-based decision can be made based on the dosage form that is formulated with the pharmaceutical ingredient, the contribution to the formulation, the source of the ingredient, i.e., synthetic, plant, animal or mineral origin, the manufacturing process for the ingredient, the route of administration of the sterile product, volume administered, risk analysis and the testing history.

A2: Would you not also consider the compendial requirement for the particular raw material as part of your risk assessment?

A3: It's a simple and broad question but you have to put some thought into it. I'm certainly not going to be able cover the subject in this forum.

It would be common practice to test raw materials for endotoxin. It's best to catch a bad raw material before it gets into a final product. That said, it's all risk and history based. Is the raw material from a vendor that you trust? Did they provide a certificate stating the material's endotoxin load? What's the risk to the final product if the raw material has an endotoxin load? What are the impacts throughout your system if a material with an endotoxin load is used in you manufacturing area? I'm sure there are other questions that need to be addressed but hopefully you see the point. You need to put some thought into it and decide what testing is required in your situation but if there is any doubt, it's best to test.

A4: Yes, thanks to LAL, it is fairly common practice now, as a part of building quality into a product, and not testing at the end to see if the product complies with the regs, as was done years ago with the RPT. With that said, you must determine what an "acceptable" endotoxin limit is for that raw material. What is the maximum concentration of the material in the final product? Is the raw material manufactured under harsh synthesis, and therefore less likely to contain endotoxin? Is the raw material from organic sources such as plants or animals, and therefore likely to contain endotoxin? This should all come into consideration when establishing endotoxin limits, based on careful risk analysis. I have seen too many instances where folks set an arbitrary limit of 1 EU/mL for a liquid raw material, yet that raw material may be in a concentration od 0.001 percent, causing the firm to discard the raw material, even though it has a negligible contribution to the overall pyro burden of the final product.

Saccharomyces sp.

I would need to know if anyone has experience in a verification of microbial counting methods for a product containing Saccharomyces spp.?

Antibiotic traces

I want to know what methodology do you use for antibiotic traces in equipments and surfaces in pharma industries. Do you know of some rapid method for this antibiotics?

-Rifaximin
-Ciprofloxacin
-Levofloxacin
-Gentamicin
- Azithromycin
-Claritromicyn
-Eritromicyn
-Rifampicin
-Trimethoprim
-Moxifloxaciny
-Nystatin
-Gentamicin + fluocinolone

A1: The challenge is HPLC methods used for potency assay of antibiotics are unsuitable as the LOD is not as important in the application of these methods compared to residue analysis.

Rapid residual measurement kits are available for beta lactam antibiotics in the dairy industry.

I believe still nystatin uses a microbial assay.

Remember cleaning validation is based on worst case studies in terms of solubility and potency and not testing for the recovery of every antibiotic so method development for all the antibiotics on your list will be unnecessary.

Specific microbiological requirements in Pharmacopoeial monographs

Had anyone ever listed raw materials with pharmacopoeial (PHEur or/and USP) monographs containing specific microbiological requirements? Or maybe anyone knows better way to prepare this kind of list than opening and reading each of hundreds of monographs? Is there any tool to do this?

A1: The USP Online has a search function so you can obtain this information.

A2: For each raw ingredient that is used in your manufacturing facility, there should be a Quality Control test specification sheet containing analytical and microbiological test requirements in which you do not have to go back and look at the monographs for a raw ingredient in a book. Someone in your organization should be responsible for generating these Quality Control test specification sheets for raw ingredients such as Quality Assurance or R&D. If there is an update in the monograph specifications for a raw ingredient, there should be some type of a document control program to generate and issue the new raw ingredient specification sheet. These raw ingredient specifications sheets can be in a book or located in a computer for everyone to have access to it.

A3: USP on-line does have powerful search functions. And, I believe, there is also some online help (tutorial) to help teach you to use advanced functions to search.

AET / PET (US/EP)

i understand that this test is not a routine test that is performed on every batch however how is the information in the chapters interpreted for USP and EP to clearly define when this test is required? i can't see it clearly stating its intent.

is it just a development test only or should it be added to routine ongoing stability and test at least one batch per year at different conditions?

Does it need to be repeated if an ingredient or primary packaging is sourced from a new supplier during the product lifecycle? Just initially or added to multiple stability timepoints and conditions?

A1: The guidance you are looking for is ICH Q6A. It basically says if you have a preservative added to your formulation you need to collect data that shows that the preservative works throughout shelf-life using the pharmacopoeial antimicrobial preservative effectiveness tests (USP <51> and Ph.Eur 5.1.3). Once you have established the preservative works, your commercial batches can be released based on preservative content measured by analytical assay. It goes on to say you should demonstrate the lowest specified concentration of preservative that works. For example, if your preservative content specification is 80-100%, then you need to collect data that shows the preservative system working at 80% to justify that lower end of your specification.

Whether or not you perform the testing while the product has been introduced to the market depends on what stability commitments you gave to the regulatory agency when the product was filed. It may depend

on how much stability data you collect during development whether or not you continue collecting shelf-life data after the final product launches.

A2: In reference to your questions, an AET/PET (USP/EP) should not be used as a routine Quality Control release test for each batch that has been produced. Instead, an analytical test such as quantitative or qualitative testing should be confirmed that the preservatives had been added to each production batch that has been produced as a routine Quality Control release test.

It is a common practice to conduct AET/PET during the development cycle of a product formulation. At first, this testing should be conducted on bulk samples of a product formulation with different preservative systems. After a preservative system has been selected for a formulation, accelerated aging is conducted on the formulation in the intended product packaging to determine if the preservative system is stable for the intended product shelf-life and whether the product packaging would have an adverse effect on the preservative system.

To confirm accelerated aging results of the product, AET/PET is conducted on real-time production samples at yearly intervals for each year that the formulation is produced. As far as the number of the batches that are used for conducting stability testing, I have seen companies use only one production batch and others will use at least three production batches. This number of batches is also dependent upon how many batches of a product formulation is produced during the year. It is usually a company-based decision on the number of batches selected for real-time stability testing.

As far as raw ingredients are sourced from a different supplier, this is a particularly good question if AET/PET needs to be re-conducted. Before a raw ingredient from a different supplier is used in a product formulation, analytical testing should be conducted to confirm that the proposed alternate source of the raw ingredient is chemically equivalent to the currently sourced raw ingredient. If not chemically equivalent, it should not be used in manufacturing. I have also seen that batches are made with the alternate source raw material to see if there is an adverse effect on the appearance and chemical attributes of the finished product. Some companies will also conduct AET/PET testing on these formulations to see if there is an adverse effect on the preservative system while others do not. From my perspective, I would recommend conducting challenge testing on the formulation containing an alternate source raw ingredient. I do not like surprises during manufacturing or during real-time stability testing.

If the chemical composition of the final packaging is changed or a different package delivery system is going to be used for a product formulation, I would recommend that accelerated aging studies be conducted on the formulation with an AET/PET be conducted at the end of the study to determine whether the packaging change had effected the preservative adequacy of the formulation. If acceptable, real-time stability testing should be conducted on production batches.

I apologize for the long answer to your posting, but the questions asked are extremely complicated.

A3: The marketed product stability protocol detailing the test parameters and time intervals are part of the regulatory submission that is approved by the agency so you can get locked in.

You can use a sunset approach committing to AET through expiry for the first or first three batches put up on stability and then relying on chemical assay.

A word of caution about presenting data that the preservative system is effective at 80% of label claim as in my experience one or more national boards of health have asked why we did not reduce the preservative concentration by 20% as we had data that it was effective. The concentration employed is governed by regulatory limits, industry practice and in-house experience with similar formulations.

Sometimes we add other challenge organisms like B. cepacia to the AET during development to get the most robust preservative system but only submit the AET data.

Re-qualification of a preservative system should be an issue around change control in making modest changes to the formulation and excipient suppliers. The use of accelerated stability studies including AET can address these concerns.

As a former stability manager I believe in the rule to assume any change will have an adverse effect unless proven otherwise.

Critical Contamination Rates & Adverse Trend Definitions

I would like to pose a question to the group about how critical contamination rates (CCR) are interpreted when defining adverse trends? For example, I understand the CCR when applying to viable samples; however, how are these rates being applied to total particulates or water/gas utility systems (i.e. tests and methods which don't trend as Growth or No Growth)? Additionally, we are struggling to come up with a working definition of "adverse trends" and would love to see what other companies have used.

Neutralizer effect in RODAC plate

I am looking for RODAC plate neutralization effect study. This study needs to be prove that content of the RODAC plate with neutralizer is sufficient to neutralize the days disinfectant which is used in clean room surface and it should not impact on recovery of the organisms from the surfaces.

This type of study is required? If not, how to justify?

Measurement of Uncertainty

How is the measurement of uncertainty, ISO 17025:2017: (7.7.1) can be applied to microbiology lab? "The laboratory shall have a procedure for monitoring the validity of results. The resulting data shall be recorded in such a way that trends are detectable …." Any practical example will be appreciated.

A1: The ISO/IEC 17025:2017 clause you quoted has nothing to do with measurement uncertainty, however, You can refer to ISO 19036 or A2LA G108 guideline for practical evaluation of uncertainty in microbiology labs.

A2: How do we implement clause ISO/IEC 17025:2017: (7.7.1) in everyday testing?

A3: Usually this is carried out by using QC standards (microbial test strains) by spiking the tested material with specific microbial load, calculate the recovery and run control chart for every type of test and ensure that the results are under control over time. In addition, Proficiency testing samples, blind samples and comparison of your results with other labs are also beneficial.

A4: A2LAG108 guidelines are suitable for microbiology lab MU calculations. Use that for reference.

objectionable

I have performed a risk analysis about our product (not sterile capsules and tablet) about objectionable organism with a low risk to find objectionable in our product.

My query is about the conclusion. If during the analysis, I identify an organism know to be pathogen for USP, I have to reject the batch?

I try to better explain. For the product I have to analyze TAMC, TYMC and E.coli and in E.coli test I find a Salmonella. My test is in specific BUT I have a pathogen in my product.

Even if the risk of proliferation is low, the TAMC and TYMC is in specific, is correct a QA/QP assessment to go on with the batch disposition?

And for raw material, water and air sample can I use the same argument?

A1: Salmonella will grow in MacConkey's broth and on MacConkey's agar but unlike E. coli does not ferment lactose.E. coli would produce acid in the broth and pink-red colonies with bile precipitation on the agar. I am not sure it would grow well at 42-44 degreeC.

It is highly unlikely to find Salmonella in a tablet or capsule unless an ingredient is a relatively unrefined plant or animal material. For example extracts from porcine intestines.

However, if you confirm the identity as Salmonella you would reject the product as Salmonella has a low infectious dose and may survive at low water activities.

Bioburden Limits for Terminally Sterilized Products

I would like to inquire as to what are the expected regulatory documents for Bioburden limits for Terminally Sterilized Products. The 10cfu/100mL would apply for aseptic products but interested in the limits for terminally sterilized products for both the US and Europe.

A1: The bioburden action level would be based on the sterilant applied within the confines of said validation approach utilized to establish the sterilization process. For example, if validated a minimum Fo of 9, then the bioburden action level should not exceed 1,000 CFUs per finished good with a bioburden alert level significantly less than 1,000 CFUs. If validated say VDmax25, would have the same action/alert level establishment approach. It would be advisable to perform microbial characterization to verify spikes in the bioburden in terms of potential resistance character based on the sterilant utilized.

Bacillus sporulating media

Can someone suggest the best medium/method for producing spores of Bacillus? There are some sporulating agars and I was wondering what works best, and which is the simplest.

A1: I've had good luck with AK Agar #2 for BS 6633 with a slight tweak of the method.

Inoculate plates and incubate 30-35C for 5-7d,
Harvest lawn with 0.9% saline and collect,
Centrifuge and resuspend pellet in 0.9% saline,
Heat shock suspension at 70C for 30 minutes then direct to ice bath,
Store at 2-8C

After determining the spore titer, dilute to whatever level you need using 0.9% saline.

Using the above method, we see the spore population hold steady for up to a year- probably longer, but we only collected validation data up to one year.

A2: The best medium I have found to stimulate Bacillus sporulation is a medium (for example PCA) supplemented with soil extract, which is made as described in Ruth Gordon's book, The Genus Bacillus, USDA Agricultural Handbook # 427, October, 1973, pp 100 - 101. Soil extract based agar was used in the publication on Bacillus acidicola, Inter J Syst Evol Micro 55:2125-2130 (2005).

Another way is to use 'sporulation salts' ... 13 g CaCl2.2H20; 10 g MgCl2.6H20; 2.8 g MnSO4; 0.5 g ZnSO4; 0.1 g FeSO4; 0.1 CuSO4 in 1 liter of 0.1 N HCl ... add 10 ml to 1 liter of medium like PCA, TSA or nutrient agar ... there are different formulations for the 'sporulation salts .. the key is the presence of divalent cations ...

A3: I suggest using AK Sporulating agar and harvesting the growth using sterile 70% IPA. You will get a stock that is 100% spores. If you do a spore stain of the stock and one harvested with sterile saline, the comparison is pretty remarkable!

A4: I've found Amended Nutrient Agar (recipe can be found in US EPA MB-15-04) and Yeast Extract Agar (recipe can be found in EN 13704 Annex A) both to be effective, simple options for spore production, with a wide range of bacterial spore forming species.

A5: I don't know if it is still available, but back in the day we used Oxoid Nutrient Agar which worked great to get Bacillus subtilis or globigii to sporulate en masse. It worked straight out of the can!

A6: TSA with a salt of Manganesium.

A7: Sporulation is common to Bacilli species yet the efficiency of the process, length of time to convert the maximum possible % under given conditions etc. are strain specific.

For "normal" Bacillus strains AK sporulation agar (available commercially) worked well in our hands.

The idea is to create a large surface area of medium and inoculate with a culture grown within rich medium. Once the culture is washed the minimal agar support some growth but the starvation and lack of nutrients lead to spores formation. This process may take up to two weeks to complete. We got 90% and up pure spore cultures by heat treatment comparison and close to a 100% based on spore staining. Temperature, inoculum concentration (cfu/sq inch), and other parameters may need optimization. You may want to examine B. thuringensis literature as the industrial production of this spore forming was optimized many years ago.

Qualification of Purchased Media

I would like to know how companies are handling qualification of purchased media for use on site? Procedurally, when using a new supplier or new type of media we would require three lots pass growth promoting quality testing and a report written. How far do companies take this? Is everyone qualifying all types from the same supplier? Ex. If I would like to purchase R2A plates from a vetted vendor who currently supplies our TSA plates, do I need to qualify that R2A as "new" or simply require it pass the routine incoming quality testing performed on every purchased lot? i.e. Test it routinely, or require 3 lots and write up a whole report summarizing the data?

A1: For each lot of prepared media that is received by your laboratory from a manufacturer, I would recommend that you conduct the following tests: Growth Promotion, Sterility, and pH. My reason for performing each of these tests is that you do not know the exact shipping conditions that the lot had been exposed to from the time of manufacturing to the time of receival at your facility. For example, was the lot exposed to high or low environmental temperatures during shipment? You want to make sure that the exposed shipping conditions of the lot did not have an adverse effect on the prepared media for the recovering or growing microorganisms. In general practice, it can take some time for a prepared microbial growth media supplier to supply three different lots of a prepared microbial growth media. I would also recommend that you find out what dehydrated microbial growth media supplier that the contract microbial media supplier is using to prepare their prepared microbial growth media batches. Do they have more than one dehydrated microbial media supplier for each type of prepared microbial growth media that they make?

If you are purchasing dehydrated media from a new supplier, I have no objections in your current practice that 3 different lots numbers of a microbial growth media need to be evaluated for growth promotion and pH to determine if the material from a new supplier is acceptable or not to your currently approved supplier. Once approved, I would still conduct growth promotion, pH and sterility testing on each laboratory prepared batch that is used within the laboratory.

Bioburden method & acceptance criteria for stopper samples

I would like to know, is there someone who is performing the bioburden analysis for the stoppers as primer packing material?

We have stopper test samples that require bioburden analysis according to the certificate of analysis, but I could not find any method at the pharmacopeia. Is there any approved pharmacopeial method for that analysis and acceptance criteria/ limits?

A1: You can use Bioburden (Enumeration Testing) by Membrane filtration method- USP <61>. However, you have to validate before performing this test for each type of non-sterile components (Droppers, Caps & Bottles) prior to sending them either for Gamm/ETO sterilization.

I believe, there are no acceptance criteria for Enumeration testing of non-sterile components. You can keep NMT 1000cfu/sample.

Just curious to know if you are using in ophthalmic products or Injectable products as requirements are different specially for injectables.

A2: You are right the bioburden of stoppers is not covered in either USP <61> or <381> Elastomeric closures for Injection.

Place 5 stoppers in 100 ml of sterile buffer in flask shake and conduct a membrane filtration count.

A3: The method we use to monitor bioburden on stoppers is derived from ISO 11737-1:2018 Sterilization of Health Care Products - Microbiological Methods - Part 1: Determination of a Population of Microorganisms on Products.

A4: What type of product will it come into contact with?

A5: Stoppers for injection have to be sterile. So I would perform a sterility test instead a bioburden test.

A6: There are two types plugs we are using for injections
1) sterile : where we do sterility test on miniature samples
2) ready to sterilize plugs : where we do bioburden test

Use of Oligotrophic Medium for the Detection of Bcc in Purified Water Samples

In 2019, there was a publication indicating the usage of a oligotrophic medium (1/10xTSA, 1x10xTSB, Reasoner’s 2nd Agar (R2A) or Reasoner’s 2nd Broth (R2AB)) which allowed for the improved recovery of Bcc organisms that are present in distilled water.

Has anyone used this above oligotrophic medium for the recovery of Bcc from purified water samples? If so, did you find an improved recovery of Bcc from water samples with this medium instead of using R2A or PCA as the recovery plate count agar or USP Chapter 60?

Reference:

Ahn, Y., Lee, UJ, Lee, YI, LiPuma, JJ, Hussong, D, Marasa, B, Cerniglia, CE. 2019. Oligotrophic media compared with a tryptic soy agar or broth for the recovery of Burkholderia cepacia complex from different storage temperatures and culture conditions. J. Microbiol. Biotechnol. 29: 1495-1505.

A1: Not for that purpose [name redacted]. I have used such dilute media (e.g. 1:10 TSB) for growth of inoculum in challenge testing to generate a more resistant challenge.

A2: Wish I had access to the paper to discuss further.

In addition to your cite, Carl Cerniglia's group at FDA has produced a number of publications in the last few years addressing cepacia complex. Wonder if this work will lead to policy development.

A comparison of culture-based, real-time PCR, droplet digital PCR and flow cytometric methods for the detection of Burkholderia cepacia complex in nuclease-free …
…, D Hussong, B Marasa, CE Cerniglia - Journal of Industrial …, 2020 - Springer

Burkholderia cepacia Complex Bacteria: a Feared Contamination Risk in Water-Based Pharmaceutical Products
M Tavares, M Kozak, A Balola… - Clinical Microbiology …, 2020 - Am Soc Microbiol

Intrinsic resistance of Burkholderia cepacia complex to benzalkonium chloride
Y Ahn, JM Kim, O Kweon, SJ Kim, RC Jones… - MBio, 2016 - Am Soc Microbiol

PDF] Effects of extended storage of chlorhexidine gluconate and benzalkonium chloride solutions on the viability of Burkholderia cenocepacia
Y Ahn, JM Kim, YJ Lee, J LiPuma, D Hussong… - J Microbiol …, 2017 - jmb.or.kr

Handling of exceedances in particle monitoring

I ask the following question, because I am interested in how the topic is dealt with in other companies or how they would proceed in that:

In our clean room class C/D we routinely perform regular measurements of the non-viable particle count (weekly). We measure cubic feet instead of cubic meters as given in related guidelines (ISO 14644-1, Annex1 EG), since a single measurement on cubic meters would otherwise take around half a Hour measure time. Our limit values are calculated down accordingly from cubic meter to cubic feet. Currently we have the internal guidance, that a deviation is created in case of an action limit being exceeded. Now the proposal of a new regulation in the SOP was made, that after the first measurement in the case of an exceedance of the action limit will lead to a further measurement. If this is again within the limits, no deviation is necessary (the exceeded value is of course also recorded for trending purposes). But if the value is exceeded again, a deviation is created. With this change the fact would be taken into account that a 1 minute exceedance in the cubic feet measurement would not necessarily have exceeded the cubic meter limit of the guideline (increased particles due to a short events).

How do you handle the particle measurement in clean room class C/D, or how would you proceed?

rFC Bacterial endotoxins

I'm writing to you to know what do you think about rFC for detection of bacterial endotoxins in pharma products. Furthermore, maybe I will make a study about this on detection of endotoxins in WFI compared with other instruments. Do you have any suggestions to how make it?

A1: It is great that you would like to use recombinant Factor C (rFC) instead of Limulus Amebocyte Lysate (LAL) from the blood of horseshoe crabs for detecting bacterial endotoxins. This way, you avoid false-positive reactions from beta-glucans and get more reproducible results from reagent lot to lot.

Generally, for you study, you best adhere to the general analytical procedure guidelines ICH Q2(R1) and USP <1225> (mostly identical) and the harmonized endotoxin test guidelines USP<85>, Ph.Eur.2.6.14 and JP 4.01. Alternatively, please, contact your local bioMérieux representative for information on our comprehensive validation support.

For some advice on comparing rFC and LAL and avoiding some misleading assumptions, you may study "LAL & rFC Comparison Study Caveats" from Kevin Williams in American Pharmaceutical Review 23(5).

A2: There have been a number of publications lately regarding this topic. Kevin Williams has one in a recent American Pharmaceutical Review (American Pharmaceutical Review, 23(5)). In addition, the July/August issue of the PDA Journal of Pharmaceutical Science and Technology (PDA JPST, 74 (4), July/August 2020) has as a review article discussing this topic. I would recommend that these would be a good place to start. It may take some time doing an old fashioned literature review to see the newer articles that may have been published between the time that the two articles that I cited and now.

A3: Charles River have recently conducted a full global water study which compares recombinant products to LAL. You may also wish to read through two recent publications in APR titled 'Functional Challenges for Alternative Bacterial Endotoxin Testing' Part One 'Attributes for Alternative Tests' was published in the May/June edition and Part 2 'Comparability' was published in the July/August edition, these will provide an unbiased overview for you on how to validate a recombinant BET product.

A4: As you are looking at evaluating rFC in Europe, earlier this year the EDQM published the new chapter 2.6.32 Test for bacterial endotoxins with recombinant factor C (rFC). You may also want to review some recently published peer-reviewed papers:

. Marius, Marine & Vacher, Frédéric & Bonnevay, Thierry. (2020). Comparison of bacterial endotoxin testing methods in purified pharmaceutical water matrices. Biologicals. 10.1016/j.biologicals.2020.07.001

. Currently available recombinant alternatives to horseshoe crab blood lysates: Are they comparable for the detection of environmental bacterial endotoxins? A Review. Jay Bolden, Chris Knutsen, Jack Levin, Catherine Milne, Tina Morris, Ned Mozier, Ingo Spreitzer, Friedrich von Wintzingerode. PDA Journal of Pharmaceutical Science and Technology Aug 2020, pdajpst.2020.012187; DOI: 10.5731/pdajpst.2020.012187

. Piehler, Maike, et. al. Comparison of LAL and rFC Assays-Participation in a Proficiency Test Program between 2014 and 2019. Microorganisms. Vol 8,3 418. 16 Mar 2020, doi:10.3390/microorganisms8030418

A5: Kevin's article gives great support for the use of recombinant technology from product compatibility perspective and detection equivalence. There is certainly a lot of great information in his article.

From a more general lab and use of product perspective know that rFC is another tool that can be used for endotoxin detection, however it does have some draw backs.
The current rFC product offerings require a fluorescence plate reader which tend to be more costly than the absorbance readers used with LAL methods. This can translate into higher startup costs.

Additionally, these readers require the signal gain settings to be adjusted for each new set of reagents. This requires running a test to determine the gain setting required for each rFC component set used. If you have multiple readers this test must be performed on each reader. Each time you receive a new kit lot the gain setting will have to be retested and set before you can test to qualify the reagent set and then test samples. This can translate into decreased laboratory efficiencies.

Furthermore, rFC reagents are all liquid and typically come in three parts, buffer, substrate and the actual rFC component that must be mixed in the proper order and ratios. Some users may find this challenging.

rFC, like LAL, is not universally compatible with all samples you might need to test. It's another tool in your box the same as LAL turbidimetric and chromogenic reagents are. Each has their strengths and weaknesses. The rFC components I have personally worked with had some issues in samples with high ionic strengths and required further dilution to overcome interference than LAL methods. Furthermore, those components could not tolerate surfactants or detergents of any kind or concentration.

One advantage of recombinant technology is the lack of response to beta glucans. If you are having problems with BG contamination in a product and BG blockers are not working with LAL then recombinant is a good technology to explore.

I would be curious to understand your driver for considering using a recombinant reagent.

A6: I think it is important for some clarity here;

· Fluorescence readers do require a gain setting for each new lot of reagents. Though rFC reagents have a shelf life of 2 years and batch sizes are large enough gain setting can be done less than once a year, and it is much simpler than running a routine assay.

· As Sean mentioned rFC reagents are liquid which is great as it means you do not need to reconstitute them. The ratio is a very simple 8:1:1 of buffer, substrate, enzyme and you only take what is needed for your testing. Also the rFC reagents are very stable(I can only talk about ours) which means you can actually make your reagents up at the start of the day and use them all day long without risk. This is very important for automation as well as you can set and leave.

· Sean`s point on surfactants and detergents is very interesting, this latest peer-reviewed paper by Marine Marius1 at Sanofi states " This latter assay(rFC) was found to be less sensitive to interference than our current assay, particularly in cleaning validation water samples"

1 - Marius, Marine & Vacher, Frédéric & Bonnevay, Thierry. (2020). Comparison of bacterial endotoxin testing methods in purified pharmaceutical water matrices. Biologicals. 10.1016/j.biologicals.2020.07.001.<https://www.researchgate.net/publication/343383901_Comparison_of_bacterial_endotoxin_testing_methods_in_purified_pharmaceutical_water_matrices>

Pantoea spp

We have identified non characteristic growth in xlda plate during mlt analysis of non sterile pharmacitical product. Upon identification it was pantoea spp.

We have reviewed articles says that it may cause opportunistic infection in immunocompromised patients but some says its just normal flora and some say its a plant pathogen But need some strong justification to release the batches. Kindly share us your valuable knowledge in this case.

A1: To evaluate the organism you should identify it to species.

A2: To help you we will need to know what type of product (oral solid, liquid, cream, etc.) and its intended use. More detailed information would also be helpful (preservatives, recommended storage, active ingredients, etc.). Otherwise we can only give more general information.

A3: Because it is a Gram-negative bacilli and an opportunistic pathogen, I would be very hesitant to release the batch. There have been many product recalls due to Pantoea (Enterobacter) contamination.

Instead of basing your decision on just my opinion, I would recommend that you follow the decision tree that is present in PDA TR 67 in determining whether this isolate is considered to be objectionable or not for your product. You must document your decision.

A4: This is the age old problem with folks finding out that some organism somewhere killed someone. While I appreciate and respect our QA brothers and sisters, a quick Google search by them will find out that an organism did just this at some isolated point in the past. Then they'll put the brakes on release of a product. For example, consider Bacillus cereus. Probably one of the most ubiquitous organisms out there. This article states: "The worst scenarios can even bring death" (https://www.sciencealert.com/here-s-why-eating-old-pasta-and-rice-can-kill-you). What?!

My advice: don't be foolish. Every organism in the world under the right circumstances can kill someone.

A5: Pantoea agglomerans is definitely an opportunistic pathogen and other species aren't Without knowing your product or the risk to the patient, there's no way to give any justification to release a batch.

A6: We are dealing with oral solid dosage forms - anti cancer drugs in regulated market.

A7: Because Pantoea had been isolated in an oral solid dosage product that is intended for cancer patients that are likely to be immunocompromised, I would recommend that the finished product be rejected.

While it is unlikely that Pantoea would proliferate in your oral solid dosage form which I assume is a tablet formulation, it can survive in the formulation. I would suspect that the source of the Pantoea contamination is from a raw ingredient that is used in compounding the oral solid dosage product formulation.

Determining whether a recovered isolate from a product formulation is objectionable or not is one of the hardest decisions that a microbiologist has to make on a daily basis. There are always going to be people that will question your decision especially after it has been determined that the recovered isolate has been decided to be objectionable. This seems to be a common occurrence for isolates that have not been identified as one of the USP indicator organisms. For this reason, I always recommend the decision tree in PDA TR 67 when making these risk-based decisions.

A8: Don’t lose sight of the fact that the recovery of a specific organism also effects the risk that other organisms that share a similar origin may also be present. All should be considered when determining product disposition.

A8: Not a serious pathogen and would not be objectionable in a solid oral dosage form.

See Front Microbiol 2019 Nov 6;10:2556.

doi: 10.3389/fmicb.2019.02556. eCollection 2019. Comparative Genomics of *Pandoraea*, a Genus Enriched in Xenobiotic Biodegradation and Metabolism

Members of the genus Pandoraea have emerged as rare opportunistic pathogens in persons with cystic fibrosis (Jørgensen et al., 2003; Johnson et al., 2004; Pimentel and MacLeod, 2008; Kokcha et al., 2013; Ambrose et al., 2016 ; Martina et al., 2017; See-Too et al., 2019) and several cases of chronic colonization and patient-to-patient transfer in this patient group have been reported (Jørgensen et al., 2003; Atkinson et al., 2006; Degand et al., 2015; Pugès et al., 2015; Ambrose et al., 2016; Dupont et al., 2017; Greninger et al., 2017). In addition to causing infection in cystic fibrosis patients, Pandoraea isolates have been recovered from blood and from samples from patients with chronic obstructive pulmonary disease or chronic granulomatous disease (Coenye et al., 2000; Daneshvar et al., 2001). Although the small number of patients involved and underlying diseases make it difficult to identify these bacteria as the cause of clinical deterioration (Martina et al., 2017; Green and Jones, 2018), one report described sepsis, multiple organ failure and death in a non-cystic fibrosis patient who underwent lung transplantation for sarcoidosis (Stryjewski et al., 2003).

A9: Are we discussing Pantoea or Pandoraea?

A10: Pantoea spp received from the identification system.

USP <60> CFU Calculation

I want to ask a question regarding calculation to be adopted for calculating number of CFU for raw material tested using less than 1g of material. Based on guidelines in USP 60, sample quantity can be reduced to

1% of the lot size if it is less than 1000mL or 1000g. If we used 215mg of material for USP 60 testing adopting this criteria how I should calculate number of CFU/g? Is it valid to use mathematical calculation to convert CFU per g although quantity used is in mg?

A1: I do not believe that any health authority will allow you to test less than 1 gram of material.

A2: USP (60) is a limit test with a passing result absence in 1 g or mL.

A3: Thanks for quick response. Just want to provide some details which I may have missed earlier. The method used for TAMC and TYMC is MF method. Entire quantity is dissolved in buffer and filtered followed by rinse which means 215mg is tested. Limits are in terms of CFU/g. If I get x colonies per 215mg should I convert values in "g" as limit is in "g"? Calculation will be Number of CFU x 1000mg/215mg.

A4: It is not possible your suggestion. If you use more than 1 g you can reduce the counts obtained to 1 g but not in reverse way.

Media reconciliation

I was asked about the concept of media reconciliation lately and, to be honest I had a limit idea of its application and still do.

From a data integrity point of view does anybody use a media reconciliation system, do they find it useful (or just an audit question dodger).

Just to clarify if anybody finds themselves in the same position as I found myself, I believe media reconciliation is a way to document the use of all of your media so that you cannot simply disregard plate results by discarding the plates. Each plate has to be accounted for.

As an example, I make 400ml of agar, and note down that I made 20 plates from this. I then go on to show that I used 10 as control plates, 6 as EM study plates and discarded 4 due to being past their allotted shelf life. This way all plates are accounted for. I don't understand the value of this process as it is all open to the integrity of the user which is the reason you are performing in the first place because you are questioning their integrity.

Please could somebody clarify the use of this system if they are performing it.

A1: A better approach is to print labels based on your EM sampling plan. Remember microbiology is not quantum mechanics.

A2: I think the inspectors are looking for gross mismatch in the number of tests being reported versus the amount of agar/media used. One way to forge that is to discard plates and another way would be to make up test data altogether. Some examples I have heard- Inspectors found out while looking at received media inventory or employee attendance data that it was impossible to have so many samples tested with so little media or so fewer employees during a given time period.

I am not aware of any firms reconciling media to the extent you have described. I firmly believe it would be counter-productive and not what inspectors are looking for.

A3: As we all know data integrity is very important and a well discussed topic. Microbiology-related data integrity is even more of a challenge for reasons that we can all understand and we are certainly coming up with improved systems and ways to prove our data.

Microbiologists have a special job and there will never be a way to remove subjectivity 100%. This is why we earn degrees and come to work every day to apply our knowledge and skills as it's not as simple or straightforward/objective as numbers/chemistry and as Tony said, it's not quantum mechanics.

Your processes and procedures should be robust enough to account for your usage of media. Each time a test is performed it is assumed that media was used, and the amount would be according to procedure. In addition, wasting valuable time writing down where each media is used is, as Samir stated, is counter-productive. How could a lab account for every mL of liquid media? Sounds as easy as keeping track of pipette tips…

If your numbers don't reconcile, are you expected to open an investigation, and how would that even be possible?

The more important issue is building a review and approval process that prevents entirely or greatly minimizes the possibility of having non-integral data. Tracking each plate does not prove your data.

In my opinion if we start a secondary documentation system/reconciliation for each media plate, we have lost sight of what data integrity means and what is value-added in respect to it.
You can say we have gotten lost amidst the trees and need to be re-focused on the forest, which is the safety and quality of our product.

So, in conclusion, I would not suggest implementing a media reconciliation system, but instead would focus that time and energy into prevention of loss of your data integrity.

A4: I'm in agreement with [names redacted] that it would be counter productive in implementing a media reconciliation for a Microbiology testing laboratory. I do not see the purpose of having a media reconciliation program. What is the laboratory trying to accomplish by having such a program in place?

From my perspective, I see some issues in having such a program. Unless the media is not autoclaved, you are not going to get 100% of the volume that had been prepared since there will be some evaporation of water from autoclaving especially if non-screwed caps are used to seal the containers for sterilization of the microbial growth media. Besides, most Petri dishes have a mark on the slide of the bottom plate that serves as a visual guide for the filling volume of Petri plates with melted agar when pouring. I do not believe that most people will use a pipet to measure out melted agar when pouring Petri dishes. In addition, pipets have a slight deviation error when measuring actual volumes. I think that it is less than +/- 1.0%.

From a data integrity viewpoint, I do suggest that sterility, pH and growth promotion be conducted on each prepared lot of microbial media and purchased ready-to-use microbial media and this data be recorded. I also think that it is important that the lot numbers of laboratory prepared and commercial microbial growth media that are used to conduct testing be recorded on the actual testing sheets for each test sample to ensure that expired lots are not used in conducting microbial testing.

As far as the comments by [name redacted], it is unfortunate that there are some bad apples in the industry who will falsify their testing. From personal experience, it is a difficult process to separate out these bad actors from the rest of us who try to do the correct thing in protecting the health of consumers. For this reason, Quality audits of the testing laboratory is the first step that needs to be conducted to try in uncovering these bad actors, but it is more difficult to uncover them if they are your own internal laboratories. In most cases of fraud, there is always some type of a paper trail or other indicators (e.g. time sheets, number of tests performed by looking at test sheets, volume of laboratory supplies ordered, volume of plates/test tubes in an incubator, etc.) present that cannot be hidden if the testing had been falsified.

EM/Bioburden QMS Software Inquiry

After performing a number of validations, risk based sample plans, and enacting a new EM program. We have a need for some QMS software to manage planning, sampling, incubations, and data integrity/ trending, etc.

I am looking to get an idea of what software others were utilizing. We currently have Labware LIMS on site but lack the microbiology module that corporate has. The cost and work to bring the module from corporate to our site seems out of reach for the time being.

We are a relatively low-risk manufacturer for diagnostic medical devices so the no patient contact and non-sterile manufacturing makes things a bit complicated as to the software application, cost, and some of the requirements that more stringent parenterals need to adhere to.

Keeping all of this in mind, i'm just curious what others are using for software?

Conductivity for purified water

i am looking to purchase purified water in containers and have ran into an issue.

The conductivity limits for the EP and USP are different. EP is <4.6 where as USP has the 3 stage approach with a <2.1 and acceptable pH as a pass.

My issue is that i have a product for sale in the US so therefore need to use purified water that complies with USP. Our current approved supplier provides PW in containers that is certified to EP with the higher allowable result. Rather than having to source and qualify/audit a new supplier for USP certified PW is there any thing else i can do to justify the use of EP water?

Could this difference have any meaningful impact on a product? Why the differences?

A1: I am not sure where your manufacturing facility is located but for drug products marketed in the US the PW must meet USP standards.

Your vendor must test and issue a certificate of analysis to document this compliance. If they cannot do that you should change vendors.

A2: A couple notes:

1. USP Stage 2 limit is 2.1 at 25°C - correct.

    a. There is no pH test if conductivity <=2.1 uS/cm.

    b. Even though the EP limit is higher, you can test the water yourself. If it is <2.1 uS/cm, you pass.

2. If conductivity > 2.1, then you measure pH and compare your conductivity (from Stage 2) to the conductivity limit in Stage 3 table.

    a. The limits are higher in Stage 3. Your water is likely to be ~5.5-6.0 pH (this is ultrapure water, then exposed to air). Your Stage 3 test could still pass USP requirements, even with your "EP water" - but you do the test yourself.

3. Could this difference have any meaningful impact on a product? From chemical perspective, realistically, there is little difference between USP and EP "Purified Water" when you purchase it in containers. Most of these waters are originally 18.2 Mohm-cm water (0.055 uS/cm) before they are packaged, but they increase in conductivity after exposure to air (harmless) and maybe extractables from container (more likely impacting TOC). This is known from experience with the production of these types of waters. But to PROVE it, that is a different challenge. Your supplier maybe could assist.

4. Why the differences? I assume you ask "why is USP and EP having different limits for PW"? The limits are same for WFI - USP developed a method and a rationale in 1996, and EP adopted same limits in ~1999.

a. USP has same limits for PW as for WFI. This is because USP applied the same "ionic impurity" rationale for WFI and PW.

b. EP took a different approach for PW, applying a chemical model/rationale differently, and thus ended up with higher limits.

c. Why apply the model differently? The reasons have never been elucidated - to my knowledge. My feeling is "this is what they are, so we must accept them."

5. From USP perspective, if the PW meets USP, it meets EP.

6. But if PW meets EP, it may or may not meet USP!

A3: Additionally in the USP conductivity chapter it has different criteria for Sterile water (PW or WFI).

For containers with a nominal volume greater than 10 mL, if the conductivity is NMT 5 µS/cm, the water meets the requirements.

If i was to use sterile EP Purified water and as long as you are detailing Sterile PW within the IND submission then i assume the higher conductivity is ok as it is comparable to USP expectation.

Strange that the higher conductivity is allowed in USP PW if it is sterilised.

is it then acceptable to use sterile EP PW (is there a designation for this as i could only see sterile WFI?) as this will be comparable with USP sterile PW?

A4: Conductivity is allowed to be higher for USP Sterile Purified Water (SPW) because the starting material to make SPW is PW, then it is suitable sterilized/packaged. USP allows for the fact that the packaging will add some conductivity. This USP SPW cond limit has been in place for >10years, and it was stolen/copied from EP which has this same SWFI cond limit. This is also described in a little more detail in USP <1231>.

If you can source a water that meets the specs within your IND, then you are ok. If you specify SPW, and your supplied water meets the requirement of SPW - ok. (Correct, there is no EP SPW, only PW in containers, not sterilized.)

Be aware. The current requirement for organic impurities for SPW is proposed to change. Currently, it is an optional test (Oxidizable substances or TOC at 8 ppm). In future, it has been proposed to change to a TOC limit test only. So source your SPW carefully.

Yeastolate- BET

I'm reaching out to experts advise on one of the issue that our lab is dealing on Endotoxins test.
Material: Yeastolate, The results shows positive on endotoxins.
Test: BET, endotoxins by standard curve. The results obtained positive, used glucan beta blocker and serial dilution.
BET test - Water standard is within expiry date.
Lysate USP lot within expiry date.

We did brainstorming session, method, training and sampling. Not a conclusive for laboratory error.

What could be the contamination?

A1: There is more evidence lately that beta glucan blocking buffers do not block ALL beta glucans when using a LAL based assay. (see LAL & rFC comparison study caveats - Williams APR V23 Issue 5).

A2: Could you please send me a link or PDF copy of the article?

A3: https://www.americanpharmaceuticalreview.com/Featured-Articles/567831-LAL-and-rFC-Comparison-Study-Caveats/

Media Validation

I need to know who to validate (if any) for determining the media (TSA) used for Microbial Limit test analysis. USP chapter (61) defines usage of 15 to 20 ml per plate for TAMC and TYMC analysis. This is asked by recent concluded Zimbabwe auditor.

A1: I assume you're pouring your own plates. Are you using a calibrated pipettor or an automated dispenser to dispense the media? If so, find those tolerances and use that as justification.

A2: But I assume that by using calibrated pipette or cylinder the chances of media solidifying and sticking would be a problem. This will then sent a lot of queries and questions in an audit

I don't know whether all the industry is following this or using this. Pls guide me.

A3: Yes, there are a number of options. Search “culture media dispenser” and you’ll find lots of options. I’ve used a bottle top dispenser and a peristaltic pump. You can keep the agar warmed and your tubing should be ok. You can clean and autoclave the tubing everyday to ensure cleanliness.

A4: Most of the ready prepared media plates are filled with 30ml of Media like TSA.

A5: I understand your question, but the guarantee of the used media is based on growth promotion, sterility tests, storage time and validity and sterilization validation.

Normally all over the world analysts prefer to dispense directly from the bottle. Because avoid a 2 contact and thus avoid possible contamination, solidify, among others ... if you want to know if you dispense correctly, doing a verification test of the number of plates against the volume of the bottle and estimates if you add the recommended.

A6: This I feel will lead to a better solution and help with a reconciliation of media. Also is there any reference of the question asked by the auditor w.r.t guidance document, white paper? Etc.

A7: I have been thinking about how to approach the subject with the auditor and I believe the following:

1. An auditor requests that can be convinced as follows:
    A. Verification and qualification of three analysts: the volume of agar served vs the number of plates.
    B. Promotion of growth by lot, having no deviations is one of thousands of indications that the volume is reliable.

Beyond that, the quality of the culture medium is important and guarantees it with the measures mentioned in my other message.

Even the volumes are indicated by the period of dryness and amount of nutrient.

Also in the majority of reference they delimit as follows

The following method is suggested for prepouring agar plates: Use automatic dispenser or pour constant amount (about 15 ml / 100 mm plate; 50 ml / 150 mm plate) of sterile agar at 60-70 ° C into each petri dish.

reference:

https://www.fda.gov/food/laboratory-methods-food/bam-chapter-3-aerobic-plate-count#:~:text=The%20following%20method%20is%20suggested,no%20higher%20than % 2010% 20dishes.

The keyword here is constant so you can do a three-analyst check, document it, and perform a statistical study of the data.

A8: I noted the other replies and just wanted to belatedly add my own thoughts.

The Approach depends on the nature of the Test Type - whether Spread Plate or Pour Plate.

In my experience setting a strict defined tolerance to agar dispense volume only makes sense for pre-poured plates for later use i.e. where automated dispensing systems can be effectively employed.

With pour plate testing, particularly where high sample volume through-put (dozens of samples being prepared from the same batch or lot) though automated systems are available, the analysts are often responsible for dispensing the agar manually and mixing the sample & agar.

I remember employing pour plate volumes of 15 - 20 ml and lab analysts become quite skilled and adept at pouring plates within this range.

The critical consideration for plate volume for these general-purpose growth media is that too little a pour may lead to sample mixing and / or dehydration issues during incubation.
One therefore needs to validate / suitability test your system (relevant incubation regimen, incubator performance, plate vent type, sample volume etc), for low level microbial recovery using a lower limit of agar volume, say 12 ml / 90 mm plate. Ensuring that analysts are trained to pour an adequate volume above this lower limit should be sufficient.
While not critical for recovery, pouring too much may lead to overfill with consequent mixing, spill, cleanliness, and potential contamination issues and obviously expense.

In the case of pour plate testing,
Above a lower volume limit at which effective sample mixing and /or dehydration effects become issues - medium dispense volume alone is not a rate limiting factor for recovery and your auditor should be clear on that.

On the other hand, pouring too much may lead to overfill with consequent mixing, spill, cleanliness, and potential contamination issues and obviously expense .

Assigning tolerance on dispense volume is a case of practicality, by all means apply a sensible tolerance for automated dispensing systems for pre-poured plates. But beware, don't just assume to apply the tolerances defined by the supplier for the relevant dispensing equipment, as these are usually determined under strict controlled conditions which are often difficult to replicate under working laboratory conditions in the Media Prep lab etc. Establish what's works for you, document it, and be prepared to defend it.

For pour plates if you have small numbers of tests you could use pipettors or sterilise media in small individual defined volumes etc etc - if it works for you and keeps the auditor happy, but it is not necessarily added value or very efficient.

However, if you have a large through-put for pour plates, manual dispensing is more efficient as long as you can establish data that the defined test system (including your preferred dispense volume) supports acceptable recovery and the analysts are well trained and follow procedure. You could even adopt a procedural post pouring visual volume check before sample prep discard and start of incubation.

Just one detail for pour plate testing techniques the temperature should not exceed 44 - 45oC for the sake of microbe recovery and the comfort of the analyst.

USP <71> testing

My question concerns sterility testing to USP <71>.

Our company has several eye products manufactured in individual LDPE resin vials. When initial suitability testing was performed the product vials were soaked in a Cidex solution of 2.4% glutaraldehyde to reduce bioburden prior to testing. All required organisms listed in USP <71> were recovered therefore it was thought that the Cidex solution did not leach through the resin and we would not get false negative results. We are changing the solution from 2.4% glutaraldehyde to a 0.6% solution of ortho-Phthalaldehyde solution. One product has had a successful repeat of the suitability with the new disinfectant. All products are made with the same LDPE resin. Can the one suitability repeat with the new solution be a justification that can apply to all other products using the resin? Or would each individual product require its own repeat suitability test?

A1: As an initial matter, why are you treating the bottles to reduce bioburden? And how are you controlling the exposure? The ophthalmic industry routinely uses terminally sterilized bottles. I assume your ophthalmic product is required to be sterile?

Next, even if you passed method suitability, we do not have enough information to understand if the study was properly performed and more importantly, whether disinfectant leaches out.

Finally, if there is leaching of disinfectant, method suitability may not work for all formulations, as the amount leached out can impact microorganism recovery, dependent on the interaction with each product matrix.

This is the first instance I have heard of using Cidex to disinfect bottles intended to package ophthalmic products.

methylchloroisothiazolinone use

I have a question about methylchloroisothiazolinone use as a preservative. I've recently seen it's use alone without the use of Methylisothiazolinone at a level of 2000ppm. Is anyone familiar with this approach? Does anyone have reference to information that can be shared about preservative performance in this format?

A1: In regards to methylchloroisothiazolinone, high concentrations of it can cause chemical burns and it is a skin and membrane irritant. Generally, I have never seen methylchloroisothiazolinone be used by itself as a preservative in cosmetic/personal care product formulations. I would believe that a usage concentration of 2000 ppm is way too high usage for a cosmetic/personal care product formulation. In combination with methylisothiazolinone, the maximum usage concentration is 15 ppm (0.15%) because both methylisothiazolinone and methylchloroisothiazolinone are both a skin sensitizer. I have seen methylisothiazolinone be used by itself in rinse-off product formulations at a concentration of 0.05 to 0.1% (48-95 ppm). However, it should be noted that methylisothiazolinone has to be used in combination with a preservative that has activity against fungi because it only has activity against bacteria. For this reason as a skin irritant, combinations of methylisothiazolinone/methylchloroisothiazolinone and methylisothiazolinone are only used in rinse-off hair care and skin product formulations.

For a reference, see David Steinberg’s book called Preservatives for Cosmetics. In addition, see if you can obtain a used copy of Kabara’s book called Cosmetic and Drug Preservation. These are 2 excellent reference books that a microbiologist should have if they are doing preservative challenge testing. Please also obtain a copy of EU Annex V for allowed preservatives in cosmetics from the internet.

A2: It’s commonly used in surfactant based rinse off cosmetic products @ 5-7 ppm active . I’ve not seen it supplied without a low level of methylisothiazolinone.

A3: Thor's Acticide 1 is a CMIT only product.

A4: I believe that Thor's Acticide 1 (methylchloroisothiazolinone) is used as a preservative in household consumer, industrial and janitorial products. Please confirm.

Furthermore, methylchloroisothiazolinone is not present by itself on the EU Annex V- Allowable Preservatives for Cosmetics. For rinse-off cosmetic and personal care product formulations, it would not be used by a cosmetic /personal care microbiologist as a single preservative or in combination with other preservatives with the exception of a blend with methylisothiazolinone at 15 ppm.

It is my understanding that the preservation of paint, household consumer, industrial and janitorial products is slightly different in what chemicals can be used as a preservative and levels from what can be used in consumer and personal care product formulations.

safe preservative levels

Where is there published information on what the acceptable safe levels for preservatives in pharmaceutical products are?
is there FDA or MHRA guidance?

I’m interested in non-sterile nasal products and specifically BKC but information on levels in general would be useful.

A1: Search this term "Preservatives Used in Pharmaceutical Industry"....I found a few websites that showed concentrations. Also, search this "Antimicrobial Preservatives Part Three: Challenges Facing Preservative Systems"

A2: I use the reference David Steinberg Preservative for Cosmetics and Phil Geis Cosmetic Microbiology which are good for topical products.

A3: The FDA does not maintain a list of approved preservatives that can be used in cosmetic and drug product formulations. However, the FDA does maintain a list of prohibited or restricted preservatives (e.g. Hexachlorophene [21 CR 250.250], Mercury compounds [21 CFR 700.13], Bithionol [21 CFR 700.11] and Halogenated salicylanilides [21 CFR 700.15]).

As far as the Medicines and Healthcare Regulatory Agency (MHRA) in the U.K., I’m not sure that they maintain a list of approved preservatives. In Europe, I do know that the European Chemicals Agency (ECHA) does maintain a list of approved biocidal products that contains preservatives. With BREXIT, I’m not sure that the MHRA will keep on following this list or develop their own list. For cosmetics in Europe, there is Annex V which is a list of approved preservatives.

I believe that Benzalkonium chloride can be used as a preservative up to a concentration of 0.1% in cosmetics. I do know that in ophthalmic products that the usage concentration of Benzalkonium chloride is between 0.01 to 0.02%. It should be noted that Benzalkonium chloride is generally sold as a 50% solution. If used at 0.1% concentration, the final active concentration of Benzalkonium chloride in a product formulation is 0.05%.

A4: I have done some searches and found articles etc but just wondering if there were any official recognised information that can be pointed to when discussing or presenting to a regulatory body.

List of Parenteral Drug Product Preservatives

I have been trying to come up with a possible list of preservatives that are currently being used to preserve parenteral drug product formulations. I do understand as to why there is a limited number of preservatives that can be used in parenteral drug products. A draft of this parenteral drug product preservative list is as follows:

Benzoic acid/Sodium benzoate

Benzyl alcohol – is a preservative that has analgesic activity that is used in intramuscular injections (antibiotics, anti-inflammatory, neuroleptics, peptide and protein products).

Chlorobutanol – is a preservative that has analgesic and anesthetic properties

m-Cresol (a Phenol derivative)– used only in antivenom products

Methylparaben

Phenol – used as a preservative in peptide and protein products

Phenoxyethanol – used as a preservative in vaccines

Propylparaben

Thimersol - limited use only in influenza vaccines

For those individuals that perform parenteral drug product formulation work, does this draft of a parenteral preservative list look correct?

Because methylparaben and propylparaben are used, is ethylparaben also being used today as a preservative in parenteral drug products? If not, why not?

Clostridium

When testing by USP <2022> Absence of Clostridium species, one of the confirmatory steps is to streak aerobically. The method specifically calls for a 48 hr. incubation. We are not in the lab on the weekends and the incubation period would be 72 hrs. Logically this would seem to be OK, since aerobic growth would not go away with longer incubation. My questions is how do we validate this?

A1: I'll remind you that chapter 2022 is a guidance/informative chapter. The last paragraph in the introduction allows validated, equivalent methods to be used: "Alternative microbiological, physicochemical, and biotechnological methods, including automated methods, may be substituted for these tests, provided they have been validated as being equivalent in their suitability for determining compliance".

If you run the test on Monday, you can have final result (assuming they are negative/no growth) on Friday. This would include the 48 hours on RCM and additional 48 hours on Columbia Agar.

You only need confirmatory tests, which would then require the additional 48 hours (aerobic and anaerobic) on Columbia Agar, should you have any recovery after the initial 96 hours of incubation.

I would see no issues if you validated the method for a 72 hour confirmatory read.

A2: You validate the shortest incubation time so adding another day of incubation is OK.

A3: Under this USP chapter for the absence of Clostridium species, may I extend an additional query on this. Since the procedure needs at least 1 g sample for each of heat and not heated, wonder what is the proper report to incude the the unit e.g. absence of Clostridium species in 2 g or in 1 g?

Antimicrobial effectiveness test <51>

I need to use a container that is not that which the product is manufactured and stored in and therefore must avoid using materials that can interact with the preservative in the containers.

My question is, can anyone explain how they would design/set up this type of experiment and what criteria they would be looking for?

A1: Before you conduct any microbial challenge testing, you need to determine the actual chemical composition of the package that you are using. It is well documented in the scientific literature in how the chemical composition of the package can affect the chemical stability of a preservative in a product formulation. For example, you can have plasticizers leach out from the packaging that are able to inactivate preservatives or have absorption of the preservative by the packaging.

This type of problem of package incompatibility with a preservative system in a product formulation is usually uncovered during accelerated aging of a product formulation in a package when conducting initial product development stability testing.

This incompatibility of the chemical composition of the package and the preservative system of a product formulation takes some time to occur to have a preservative challenge test failure. It is not immediate.

A2: Incompatibilities of the primary packaging is determined using extractable/leachable and stability testing.

The AET may be conducted on a composite sample to provide sufficient volume in a neutral container like a glass vial.

A3: Adding to previous comments - one should also consider surface to volume established by the intended primary product package.

FDA In Vitro Microbial Kill Rate Study for Ophthalmics and Antibiotics

Has anyone performed an FDA In Vitro Microbial Kill Rate Study for Ophthalmic solutions? Specifically, I am looking at options for neutralizing tobramycin on agar plates and/or in the dilution tubes.

A1: Have you tried Sodium polyanethol sulfonate?

A2: It is my understanding that Clavulanic acid is able to inactivate Tobramycin. Clavulanic acid is a beta-lactamase inhibitor. However, I do not know the concentration of Clavulanic acid that is able to cause this inactivation of tobramycin. The reference below might be able to help you further.

Reference:

Flourney, DJ Factors influencing the inactivation of aminoglycosides by beta-lactams. Methods and Findings in Experimental and Clinical Pharmacology 30 Sept 1979, 1 (4): 233-237.

A3: A long shot, but back in a very dim & distant past various concentrations of NaCl were used to neutralise aminoglycosides for antibiotic plates assays where the other agent of a combination that included aminoglycoside was being assayed. NaCl supplementation of Mueller Hinton Broth also causes increases in observed MICs for aminoglycosides. I guess there are likely more effective neutralisers in use these days, and the difficulty with NaCl would be ensuring that the effective NaCl concentration did not itself inhibit recovery of the strains of the indicator species being used.

Raw Material Testing for Sterile Pharma

While objectionable organisms should be tested for (USP <62>) for Raw Materials for non-sterile products, I have found disagreement for sterile products. Some people feel we should test for USP <61> only for RMs (without a USP monograph) for steriles (e.g., T. Cundell), while others feel we should test for both <61> and <62>.

Comments and references requested please.

A1: This is from the CFR that Tony has quoted many times:

"TITLE 21--FOOD AND DRUGS

CHAPTER I--FOOD AND DRUG ADMINISTRATION

DEPARTMENT OF HEALTH AND HUMAN SERVICES

SUBCHAPTER C--DRUGS: GENERAL

PART 211 -- CURRENT GOOD MANUFACTURING PRACTICE FOR FINISHED PHARMACEUTICALS

Subpart F--Production and Process Controls

Sec. 211.113 Control of microbiological contamination.

(a) Appropriate written procedures, designed to prevent objectionable microorganisms in drug products not required to be sterile, shall be established and followed.

(b) Appropriate written procedures, designed to prevent microbiological contamination of drug products purporting to be sterile, shall be established and followed. Such procedures shall include validation of all aseptic and sterilization processes."

I think the title needs to be tied to the description. This is the chapter/part regarding "Finished Pharmaceuticals" so if it's a non-sterile "finished" drug, it needs to be tested for objectionable organisms. If it's a "finished" sterile drug, it needs to be sterile, no mention or objectionable or otherwise. Since the raw material isn't finished product, I don't think it needs to be tested and ALL organisms in a sterile finished product would be considered objectionable. Since the final fill/finish will remove the bioburden that's present, the total bioburden is what "counts".

A2: I've heard this as well but this is what I have advised clients - For sterile products, RM testing may be necessary to ensure that certain components, especially aqueous RM or those derived from plant or animal sources, are not grossly contaminated prior to use in the drug product formulation. Non-aqueous RM should be eligible for a reduction or elimination of testing based upon a risk assessment. But all microorganisms are considered objectionable in sterile products and the sterilization process is validated to remove or prevent the introduction of microorganisms in the finished product.

Because non-sterile manufacturing processes are required to exclude or eliminate certain organisms (i.e., objectionable organisms) but not all microorganisms from the finished product, USP chapter <62> provides advice for how to test for specified organisms indicative of the presence of objectionable organisms in non-sterile formulations. The presence of USP <62> organisms in RM used for sterile drug products does not necessarily indicate a GMP issue because small numbers of these organisms will not affect the quality of the raw material or finished product (e.g., through API/excipient degradation or the contribution of toxins) if adequate pre-sterilization controls are implemented. Because the validated sterilization process removes or kills these organisms, they pose no risk to the patient. However, large

numbers of microorganisms in sterile product RM or bulk drug product solutions could signal GMP issues and affect product quality.

In addition, 21 CFR 211 only references objectionable organisms with regard to non-sterile products (211.84(d)(6), 211.113(a) and 211.165(b))

A3: I look at a bioburden analysis as a determination of both the number of microorganisms and their ability to withstand the sterilization process.

For example, for a sterile filtration process you may be concerned with the size of bacteria while with a steam sterilization process you would be interested in heat resistance of bacterial spores. The identity of the isolates may provide information related to this risk.

This can be determined by measuring the size of vegetative cells or conducting a biological test for a spore-former.

I see that the <62> test for specific microorganisms has little or no value.

A4: For sterile materials hoy should test for usp 71 (not 61).

autoclave data integrity

I want to know if there is any requirement for data integrity for autoclaves?

A1: The probes and timer is calibrated and a hard copy printout becomes part of the batch record or retained in an electronic file.

A2: There is no separate guideline for data integrity issues for autoclave. Please follow standard regulatory Data Integrity guidelines as per requirement.

CCIT for sterile multidose ophthalmic containers

We are conducting a CCIT container closure integrity test method for ophthalmic multidose plastic containers, we have chosen the *Dye Ingress* *method *with a Methylene Blue 0.1% aq. solution.

We are still searching for the proper vacuum pressure level for these types of containers.

There are some defined Pressures and Times in legislation, but for rubber containers (commonly lyophilized vials):

"USP <381> and Ph. Eur. 3.2.9 (8, 9) cite a relative vacuum pressure of –27 kPa as an appropriate test pressure. ISO 8362-5 Annex C quotes a similar test pressure of –25 kPa. USP 31 <381> and Ph. Eur. 3.2.9 recommend application of vacuum pressure for 10 min, and ISO 8362 (10) has an application time of 30 min."*

We would kindly appreciate some information about this topic.

USP Chapter 51 Initial Inoculum Concentration

I am wondering how people are interpreting the USP <51> requirement for initial inoculum concentration for antimicrobial effectiveness testing.
The criteria is stated as "between 1 x 10^5 and 1 x 10^6 CFU/mL". Notice there are no decimals there.

When dealing with such high concentrations and exponents are used, there is usually a decimal to indicate approximate count because there is only so much rounding that is appropriate in microbiology, especially when performing serial dilutions.

With that being said, is this being interpreted as 'not less than 1.00 x 10^5 CFU/mL' and 'not more than 1.00 x 10^6 CFU/mL', which would be a literal interpretation, or are we only concerned with the exponent?

For example, if my initial concentration is calculated as 10^5 x 10^6 CFU/mL and rounded to 1.1 x 106, would this call for a re-test or is any leading number in the 10^5-10^6 range acceptable?

A1: I would refer you to the rounding rules in USP General Notes.

A2: I do agree with [name redacted] that you should be following the USP rounding rules when it comes to the calculated inoculum counts in a finished challenge test product sample.

If the calculated microbial count is between 1.0 and 1.5 x 10e6 CFU/ml or gram, I would recommend that it be rounded down to 1 x 10e6 CFU/ml or gram. From a practical point of view, it doesn't matter if the microbial count is between 1.0 and 1.5 x 10e6 CFU/ml or gram because I would expect that you are going to see the same amount of reduction in the microbial counts by the preservative system if cidal or inhibitory.

When the calculated microbial count is less than 1.0 x 10e5 CFU/ml or gram, I'm not that comfortable in rounding up depending how far off you are from the minimum inoculum level of 1.0 x 10e5 CFU. If your calculated microbial count is between 9.0 and 9.99 x 10e4 CFU, I would not have an issue in rounding up to 1 x 10e5 CFU/ml or gram.

If your calculated microbial counts are less than 9.0 x 10e4 CFU/ml or gram, I'm not that comfortable in rounding up because there is an issue in conducting the preparation of the challenge test inoculum that needs to be corrected. There was either a procedure dilution error or the harvested turbidity reading is incorrect for an inoculum challenge test organism.

When it comes up to the decision of rounding down or up, it is always an issue with the non-microbiologist auditors if you do not have a USP Rounding Standard Operating Procedure for the laboratory or it is not stated somewhere in your challenge test method when it comes to calculating the inoculum count in a finished product challenge test sample.

A3: I just realized that the text in my original question did not format properly and so my question may not have been clear.

As the USP rounding rules seem to apply to numbers using decimal places as part of the values, these rounding rules have not been helpful to me.
When we write 5.0 x 10^5, it's just an abbreviation to the actual number which is 500,000. I could use rounding rules on decimal numbers only. I don’t think there are rounding rules for whole numbers on such a high scale.

I'm going to re-post my question using carets to indicate my exponent values. Maybe it will make more sense. Please reconsider the question.

Viruses/Bacteriophages

I work at a company that produces (separate plants) SODF, Oral preparations - liquid and semi-solid forms, disinfectants and so on… We just started with disinfectants (guess why) and we are implementing EN standards for its efficacy on bacteria, fungi, yeasts and viruses.

Question 1: During method verification, what parameters should we include (we planned precision), how many batches of the product should be tested for validation and do you recommend more then one analyst?

Question 2: We test non-sterile solid oral dosage forms in the lab already, and we have separated labs for that and growth promotion test. Can we work with viruses in the same lab where we are working with other microorganisms or is there some guideline advising not to do that? We have BSC II, and negative pressure in that area, airlocks, showers (basically at least BSL 2 laboratory).

A1: Because you are using the EN methods, I suspect that the location of your company is outside of the United States. In addition, I suspect that you are performing testing on your disinfectants for registration purposes instead of disinfectant qualification for usage in a manufacturing plant. Please confirm because it is a different process for each in how many batches that need to be tested. If you are trying to qualify a disinfectant for usage in a manufacturing plant, it is not a usual practice to perform disinfectant qualification for virucidal activity.

For conducting virucidal testing for a disinfectant, it is usually performed in a separate room that contains a biological safety cabinet to prevent the introduction of bacterial contamination into the cell cultures. This is the reason as to why antibiotics such as gentamicin are often used as an ingredient in the cell culture medium for the propagation in which the cells are used in performing virus plaque assays.

When I used to perform virus research testing, it was a strict rule that conducting virus and bacterial testing not be performed in the same room in order to prevent contamination of the cell cultures no matter if a biological safety cabinet was present. I also found that it requires a different skill set in regards to the degree of aseptic technique to prevent the bacterial contamination of the cell cultures that is more stringent than working with bacteria. Before everyone gets upset, this is just my opinion.

A2: Yes, it's in Europe and we are performing tests for registration purposes. My colleague wants to do the method verification with two analysts, two replications on three batches for bactericidal testing. This is a lot of work, and we could really use some guidance on this topic.

At the moment, we are just investigating if we even have the conditions for working with viruses. Especially because we are already working according to GMP/GLP for testing the non-sterile products.

We have a spare laboratory (grade D, with BSC II) that we could use for working with cell lines, to separate it from living microorganisms, but it has a positive pressure to the corridor.

A3: To perform the necessary testing for a disinfectant registration for a product formulation, it is always a labor intensive and time consuming process.

As far as the number of batches to be tested for European registration purposes, I will admit that I’m not familiar with the number of batches that need to be tested. For the number of batches to be tested for a European disinfectant registration, I would suggest that you directly contact the European Chemicals Agency. For disinfectant registration purposes in the United States, the number of batches to be tested is indicated in an E.P.A. document called E.P.A. Product Performance Guidelines Office of Chemical Safety and Pollution Prevention (OCSPP) 810.2200: Disinfectants for Use on Environmental Surfaces, Guidance for Efficacy Testing. Depending upon the type of antimicrobial claim being made for a product, the number of batches to be tested ranges from 2 to 3 different batches that need to be tested on separate days.

As far as conducting testing for a European registration, I would suggest that you also obtain a copy of EN 14885 - Chemical disinfectants and antiseptics. Application of European Standards for chemical disinfectants and antiseptics. This document specifies which European standard tests that will need to be performed to support various antimicrobial claims for a disinfectant. For your information, bactericidal, yeasticidal and fungicidal testing, qualitative suspension testing with need to be conducted on microorganisms in both a suspension and on dried surfaces. There is no such requirement for virucidal testing.

EM to bring an area back up

What EM and water testing would you recommend after a few month shutdown? Our gowning practices were reduced to just scrubs and we use an isolator for filling. We did not sample for EM or water during the shutdown. The only major changes were new floors in Grade C and D and a degasser added to the WFI still.

A1: Once it's all cleaned and purged with air changes, I would perform EM and compare those counts to your previous trending counts. If it's all within your previous trending levels, you should be good to go. Since you're filling in an isolator, your risk is lower, though not zero, of environmental contaminants getting into your product.

The water may be a different depending on your circulation. Has your water been circulating at high temperature? You should still perform water samples prior to production.

A2: A degasser is usually added to WFI systems in which a still is used to produce water to remove dissolved carbon dioxide, volatile organics and oxygen. With the presence of a degasser unit in a WFI still system, the conductivity of the water should be lower with it than without it. Generally, WFI that is produced by using a still are hot water circulating systems. Your question concerning whether what type of microbial testing should be performed is a very good question. If you were having issues in obtaining the WFI water conductivity readings (e.g. *<* 1.3 µS/cm at 25C [USP} or *<* 1.1 µS/cm at 20C (EP bulk)] without the presence of the degasser unit, I would think that you would need to conduct re-validation of the WFI system because the chemical characteristics of the water had been improved. Because dissolved carbon dioxide is removed by the degasser unit, I also suspect that the Total Organic Carbon would be lower because TOC instruments measure the amount of carbon dioxide in a sample. I will admit that I do not know how to justify not conducting re-validation of the system by the addition of a degasser to a WFI still system because it would cause a major improvement in WFI conductivity.

In regard to your question about air sampling in which the floors of C and D areas had been changed, I would assume that the HEPA filters had also been changed after the new floor installation. The removal and installation of the new floors could have generated particulates that might have clogged the HEPA filters that are present in these rooms if the air is re-circulated to some degree. There should be no reason to conduct an EMPQ study since the air sampling sites would still be the same. However, I would conduct particulate counts of these C and D areas to ensure that they are meeting the particulate levels with the presence of the new floors. As far as microbial sampling, I would not change the frequency or the type of air microbial testing from what had been conducted in the past.

Surface sampling technique

I am performing an informal market survey on how analysts perform surface contact plate sampling. In the recent update of the FDA micro guidance the method suggested appears to be a vertical up/down sampling style however in my experience many sites perform a roll on- hold- roll off method. The mechanical contact plate sampling devices also perform a vertical sample with 0.5kg of pressure and a hold for 10 seconds.

Is anyone aware of any technical literature to study the different methods and draw any conclusions?

Finally would you respond on which method is favored in your site to get a straw poll of which method is the most common among PMF readers?

A1: The ORS document is quite subjective in their description, which reads as follows: "Gently but firmly touch the RODAC agar surface against the area being sampled, exert moderate, even, vertical pressure and then carefully replace lid".

You definitely want to roll the agar across the surface in order to ensure even distribution of the agar across the sampling surface and to aid in the ability to "lift" microorganisms from the surface, if present.

If you apply a contact plate perpendicular to the surface (i.e. you attempt to contact the entire plate surface without rolling) you can, and likely will, create an air gap/break between the contact plate and the sampling surface.

This can easily be demonstrated by operators attempting it both ways. I strongly recommend trying this against a dark surface for visualization purposes.

While I won't say that you cannot take a sufficient sample using either method, certainly the "rolling" procedure would be a best practice. Looking forward to the other comments.

A2: I think it depends on the shape of the item you're plating.

We perform the rolling method on surfaces that aren't flat. If a surface is flat, I'm not so sure it matters as long as there is no air bubble trapped preventing the item and the agar from coming in complete contact.

A3: I think you can interpret this as - the rolling portion should be done as the plates touch starts and then "exert moderate, even, vertical pressure" when the plate has complete contact, followed by rolling off and cover with lid. I've never ever seen direct up and down contact plate application.

A4: The Standard Methods for the Examination of Dairy Products 13.044 RODAC Plate Method cites literature comparing the plate method with swab contract method describes the rolling uniform pressure technique, I consider this the defining standard method.

RVS sterilization as per Indian pharmacopeia & United state pharmacopeia

We are analyzing salmonella/10 g test in non sterile product as per the Indian pharmacopeia (IP-Chapter 2.2.9) & United state pharmacopeia (USP-Chapter 62) both. We are using enrichment medium-RVS broth (Rappaport Vassiliadis Salmonella Enrichment Broth) during testing. IP recommended RVS sterilization at 115ºC for 30 mins where as USP recommended RVS sterilization as per validated cycle.My question is that can we use RVS which is sterilize at 115ºC for 30 mins in USP testing?

A1: USP general chapter <62> had provided "solution and culture media" for information, meaning the information there can be used as a guide to prepare the culture media. The requirements to use the culture media for USP <62> testing are to ensure that the prepared media has a capability to promote the growth as intended in the test method, and have not lost the growth promoting ability after sterilization. This is demonstrated by Growth Promotion testing for each culture media as outlined in the chapter before using them in testing or method validation.

So to answer to your question: you can use RVS which is sterilize at 115ºC for 30 mins in USP testing, if the prepared media passed the USP <62>Table 1. "Growth Promoting, Inhibitory, and Indicative Properties of Media".

A2: Have you validated any autoclave cycles? Most likely that cycle would be fine but you should validate your autoclave using the maximum amount of media you would autoclave and show the media is fertile over the course of time. You should also measure pH and appearance. Our RVS sterilization recommendation is 115C for 15 minutes. Please reference USP 1117 for information on validating an autoclave cycle.

A3: We had already validated our autoclave cycle of RVS (115ºC for 15 mins) with maximum load of media.In routine, we are sterilizing RVS at both 115ºC for 15 mins & 115ºC for 30 mins but there is no change in pH of media as well as physical appearance of medium.

Bio indicators for sterilization processes

We are using validated Bio indicators for sterilization processes to ensure there are no viable microorganisms.

We found that the following occurrence happened for sterilization process performed in our facility:

The sterilization process Bio indicators were used before their expiration date passed. But, the growth evaluation performed after the Bio indicators expiration date passed.
Growth evaluation test has passed ensuring the sterilization process was performed adequately.

Is there a problem relying on the growth evaluation results received?

A1: This is not OK. Even though there were viable spores recovered from the BI, there is no guarantee the resistance of the spores still met the specs (i.e., D-value).

A2: Using BIs prior there qualification could be considered proceeding at risk. As you used them within expiration and the qualification passed you collected valid sterilization process data.

However, the FDA does not care for this type of practice as companies may be tempted to use the data even if the qualification failed.

A3: There are big problems should you choose to rely on the growth evaluation results received. Those results are indefensible and create a false sense of sterility.

A4: I misread that the BIs were exposed after the expiration period. You can argue the results are acceptable since the BIs were exposed prior to expiration.

A5: I would say that you could use the results to allow you to proceed at risk, but you should repeat with a fully compliant test as soon as possible.

Regarding internal quality check

We perform microbiology assay of various antibiotics as per USP, IP and BP.

If two analyst perform test of assay of same antibiotic, What should be criteria of result in both analyst. How many difference is acceptable for both results.

Recall of Cottonelle Flushable Wipe For P. Gergovaie Contamination

PSA about Cottonelle flushable wipes recall for bacterial contamination. Check this website if you have any. It will ask for a lot # that looks something like this:

BI020505X-02 07:13

Do not use these as they may be contaminated with a potentially problematic bacteria (Pluralibacter gergovaie - formerly Enterobacter gergovaie).

https://www.cottonelle.com/en-us/recallfaq

I wonder what their AET results looked like?

A1: Your question concerning AET results for the recall of Cottonelle flushable wipes for the presence of Pluralibacter (Enterobacter) gergoviae is an exceptionally good question. I believe that the preservative system in this recalled wipe product is Caprylyl glycol and Sodium benzoate.

To determine preservative adequacy of the flushable wipe product formulation, challenge testing on these types of products is a 2-part process. First, you would have to conduct microbial challenge testing of the solution itself that had been used to impregnate the wipe or nonwoven substrate. A second challenge test would then have to be conducted on the impregnated wipe solution and nonwoven substrate (the wipe itself). I am sure that a USP AET challenge test or something that is similar to this challenge test method had been conducted on the solution that had been used to impregnate the wipe or nonwoven substrate.

For this type of flushable product, a biodegradable fiber is often used to make the wipe, and the composition of these wipes will consist of cellulose or a viscose fiber-based material such as pulp, bamboo or cotton. It is a well-known fact that preservatives can be inactivated by cellulose and cellulose derivatives. It is known in the literature that Sodium benzoate could be absorbed by cellulose making it unavailable as a preservative. In conducting a challenge test on the impregnated wipes, the USP AET challenge test method would not be appropriate. To detect whether a wipe formulation (e.g. impregnated solution and nonwoven substrate) is adequately preserved due to the incompatibility of the wipe composition with the solution, there is no compendial challenge test method. In Chapter 22 of the PCPC Microbiology Guidelines, several challenge test methodologies for wipe product formulations are indicated.

Besides in having questions as to whether this wipe product formulation is adequately preserved, I do not understand how Pluralibacter gergoviae had not been detected when microbial content testing had been conducted on the various tested batches. What is the source of the contamination? From my experience, it is highly unlikely that the source of microbial contamination was the wipe itself. Nonwoven substates used as the wipe are more likely to be a source of mold contamination in these types of products. Because the Pluralibacter gergoviae had been identified as the recalled organism, it is highly likely that the source of this organism is water that had been used to prepare the solution that had been used to make the impregnated solution or water that had been used in cleaning and sanitization of manufacturing equipment.

Time Tolerance for subculture incubation

Kindly advice what is the time Tolerance for subculture incubation (± how many hours) for the below incubation condition, transfer 1 mL of Soybean-Casein Digest Broth to 100 mL of MacConkey Broth, and incubate at 42° to 44° for 24 to 48 hours((± how many hours)?

For example

If the sample is pulled out for the subculture after 50.5 hours ? instead of (24-48 hours) could the analysis being accepted?

pH of Soybean–Casein Digest Broth

The pH for Soybean–Casein Digest Broth is 7.3 ± 0.2 at 25°.

When we add neutralisers (lecithin and Polysorbate20) to this broth when it is prepared and then sterilised i am finding that the pH is dropping to the bottom value of 7.1 and sometimes even 7.0. If all growth promotion testing is successful but we are consistently obtaining pH below the stated Soybean–Casein Digest Broth range how can we justify allowing a range for when we prepare and through its shelf life of a slightly lower range e.g 6.8 if all GPT results are satisfactory?

The additives that people add for neutralisation don't seem to be accounted for in the final pH shift. or is it expected that i adjust the pH up slightly prior to sterilisation?

A1: We adjust the pH prior to autoclaving. You would have to perform some trial runs to determine how much to adjust pre-autoclave. We performed toxicity studies with this method with no issues.

A2: Yes, this pH drop is common after sterilization. In reality, bugs don't know the difference between 7.1 and 7.0...but this is the intersection of science vs regulations. The spec exists and the process should be controlled to meet that spec.

A3: In such case it's advisable to adjust the pH in pre-autoclave conditions so that the drop is compensated.

A4: In reference to this posting, I'm going to play the role of devil's advocate.

It is true that by adding Polysorbate (Tween) and Soy Lecithin to Soybean-Casein Digest Medium that you are going to change the pH of the medium because you have changed the chemical composition of Soybean-Casein Digest Medium so that the pH will no longer be at 7.3 +/- 0.2 after autoclaving. Furthermore, it is often common to see a pH drop in all sterilized microbial media after autoclaving. As far as adjusting the pH before autoclaving, I do have questions if this is an acceptable practice because how do you know what is going to be the pH drop of the batch after autoclaving. I can see adjusting the pH in each of the batch containers after autoclaving by using sterile HCL or NaOH solution because the manufacturer has a recommended pH range for a non-modified version of the media. However, I would find this practice in adjusting the pH after autoclaving to be somewhat labor intensive. Furthermore, I do question as to whether the adjusted pH of the batch would be justified because you have now changed the actual chemical composition of Soybean-Casein Digest Medium with the addition of preservative neutralizers such as Polysorbate and Lecithin. I do not see how you can justify the use of Soybean-Casein Digest Medium pH range for Soybean-Casein Digest Medium with Polysorbate and Lecithin because the chemical compositions of these two enrichment broths are now different.

If you are preparing a batch of Soybean-Casein Digest Medium with Polysorbate and Lecithin, I would think that you have two options. One option is not conduct a pH reading of the batch after sterilization and use growth promotion data to justify the usage of the media because the medium is no longer Soybean-Casein Digest Medium that complies with the chemical specifications from the manufacturer due to the addition of preservative neutralizers. I would think that a better second option is to record the pH of Soybean-Casein Digest Medium with Polysorbate and Tween batches and develop your own in-house internal pH specification and have growth promotion data to justify the usage of this modified enrichment broth. In addition, I would suspect that batches from different manufacturers of Polysorbate and Lecithin may have an affect on the pH of this medium in which the justification of an internal pH range may also have to be periodically adjusted.

A5: I'm of the opinion that it makes sense to hold TSB plus neutralizers/surfactants to the same pH as standard TSB. If you both purchase pre-made and make your own media, it's more manageable to have a single pH range for that media type instead of two separate requirements for purchased versus prepared. Every single media vendor we use (Hardy Diagnostics, BD, Remel, bioMérieux) that sells TSB + polysorbate and lecithin has set pH specification at the same 7.3 ± 0.2 as standard TSB. The same goes for TSA with polysorbate and lecithin (RODACs for example), pH is set identical to standard TSA.

On the matter of pH adjustment of media being prepared in the lab, I much prefer to do the pH adjustment before sterilization as the best practice to maintain consistency of the media throughout the batch and maintain sterility. For example, for media dispensed into tubes or separate bottles which are then autoclaved, if pH has drifted out of range after sterilization then each individual tube or bottle would require pH adjustment. This could add some variability since each container may be a little different depending on the amount of NaOH or HCl added and it also increases the risk of contaminating the media by open each container for pH adjustment.

There are some media that are definitely moving targets with regards to pH and sterilization. R2A and SDA come to mind as well as TSB with polysorbate and lecithin. For media that have significant pH drift, our procedure identifies those media and alerts the preparer to plan ahead for a certain amount of pH drift after sterilization. It works well and the pH drift is predictable. If you know ahead of time that a certain medium typically drops 0.2 pH units after sterilization, you might want to ensure your pre-sterilization pH is bumped up 0.2 from target before autoclaving.

A6: Our TSB +LT has a similar pH range of 7.1-7.5, so while you're technical correct about TSB with LT not being the same formulation as TSB, the resulting pH range is identical from a quality standpoint. It's pretty common to adjust the pH a little higher prior to sterilization.

This is from our Merck Manual:

"In general it is not necessary to adjust the pH of a commercially available culture medium. Dehydrated culture media have typical compositions and the pH may have been adjusted to suit the performance. For culture media prepared from individual components, a pH adjustment may be necessary. The pH should be adjusted so that after sterilization and cooling to 25°C the medium has the required pH ± 0,2pH units unless otherwise stated in the manufacturer's instructions."

A7: According to my understanding, your TSB with LT is actually TSB with 0.07% Lecithin and 0.5% Tween. For conducting sterility testing, I have no issues with your TSB with 0.07% Lecithin and 0.5% Tween or TSB with 0.1% Tween in meeting the pH specification of TSB itself due to the low concentrations of Lecithin and Tween. Furthermore, it is more likely that sterile products to be tested will be by using a membrane filtration method. With the usage of low concentrations of Tween and Lecithin in a TSB enrichment in conjunction with membrane filtration, it would be very easy to demonstrate method suitability by recovering inoculated organisms.

When it comes to conducting microbial testing of non-sterile product formulations, it is a different ball game for preservative neutralization because a lot more of different types of preservatives are being used in comparison to the limited number of preservatives that are commonly used in formulating sterile products. By using low concentrations of Lecithin and Tween (e.g. <1.0%) in an enrichment broth, it is highly unlikely that the antimicrobial activity of most commonly used preservatives in a non-sterile product would be neutralized. In E.P 2.6.13 and the ISO Cosmetic methods in which non-sterile products are being tested for microbial content, they recommend the usage of higher concentrations of Polysorbate 80 (e.g. 30 g/Liter) and Lecithin (3 g/Liter) than what your company is providing for the analysis of sterile products. From my experience, I do see that most people are using TSB with 4% Tween 80 and 0.5% Lecithin as an enrichment broth in the microbial analysis of non-sterile products.

By using a higher concentration of Polysorbate 80 (e.g. 30 g/Liter) and Lecithin (e.g. 3 g/Liter) in TSB, I do not see how that these concentrations would not affect the pH of TSB that is not in the pH range of 7.3 +/- 0.2.

From my experience in performing the analysis of sterile and non-sterile product formulations, assumptions that are commonly used for conducting sterility testing of sterile products can not be used for the microbial content analysis of non-sterile products. The usage level of Polysorbate 80 and Lecithin in TSB is one of them.

Suitabilty USP <61>

We are having an issue with a client's product. When plating the Aspergillus on SDA we are getting extremely low counts but getting normal counts when plated on TSA. Also is it normal to run Suitability in triplicate (running the full testing three times?) We normally run suitability just the once with indicated neutralizers per USP <61>, plating in duplicate of course.

If you achieve growth on TSA but not SDA for a TYMC can you report the TSA or does the suitability fail?

Also should suitability reports be done in the same manner as those for say an HPLC method validation to include accuracy and linearity as well? If not why not?

A1: Is this commercially prepared, or in-house SDA? Also, how old are the Aspergillus culture and is it a spore suspension or sub-culture? Did you try the initial growth on PDA as well? I would say no, you can't count the TAMC as your TYMC because there's something going on with the ability of your cultures to grow on SDA which needs to be investigated.

A2: Supposing you are performing a plate-count method suitability, I will try a further dilution, for example a 1/100, since there is not a proper recovery in SDA, this is justified.

About accuracy al linearity, pharmacopeia states to comply with a Factor 2 between the mean of your validation result and your inoculum recovered without the product tested.

Method suitability is performed three times, if not possible with three different batches, it's an option to do it by triplicate with the same batch.

Factor 2 should comply by comparing the results of the three validation results.

A3: Did you rule out if there was an issue with the SDA batch? For example, did you perform growth promotion testing on SDA before conducting suitability testing by using Candida albicans ATCC 10231 and Aspergillus brasiliensis ATCC 16404 with inoculums of less than 100 CFU? You need to demonstrate recovery of both Candida albicans and Aspergillus brasiliensis by using SDA for suitability testing. If you are getting the correct amount of recovery by using TSA, I suspect that there might be an issue with SDA.

Antibiotic assay

We perform microbiology assay of various antibiotics as per USP, IP and BP.

If two analyst perform test of assay of same antibiotic, what should be difference criteria of result in both analyst? How many difference is acceptable for both results? As in chemical assay the acceptable difference is 1 per cent.

A1: The duplicate testing was performed by error or intentionally as per your method?

If your method/SOP requires duplicate testing, acceptance criteria regarding difference between two analysts should be a part of your SOP- considering high variability of bio-assays, I have seen +/- 2 SD and remaining within your spec range (90-110 or 90-120%).

If duplicate testing was performed by error, you should report average as long as both values are in spec. An investigation should be performed and CAPA to ensure it doesn't happen again because the antibiotic bio-assays are highly variable and you want to avoid generating unnecessary data.

Burkholderia cepacia complex Contamination in Cosmetic/Personal Care Products

Based upon the paper by L. Jimenez in American Pharmaceutical Review (October 2019, Vol.22, Issue 6), he showed that Burkholderia cepacia had been responsible for 102 recalls of non-sterile drug products during the period of 2012-2019 (e.g. an average of 13 product recalls/year) which is nearly 48% of the recalls for non-sterile drug products.

In November 2019, USP Chapter 60 – Microbiological Examination of Non-sterile Products-Test for Burkholderia cepacia complex became effect for the testing non-sterile drug inhalation and aqueous preparations for oral, oromucosal, cutaneous or nasal use. With the implementation of this chapter, hopefully the number of product recalls for Burkholderia cepacia complex in non-sterile drug products will be reduced.

Generally, CDER of the FDA is responsible for regulating drug product products. CFSAN of the FDA is responsible for regulating food, dietary supplements, and cosmetics (Personal Care Products). For the microbial testing of food and cosmetic products, there are test methods for the analysis of these products in the FDA Bacteriological Analytical Manual (BAM). CFSAN is responsible for the FDA Bacteriological Analytical Manual (BAM). If a cosmetic or a personal care product is an Over-the-Counter (OTC) drug

product based upon an FDA Over-the-Counter (OTC) Product Drug Monograph, the product comes under the responsibility of CDER.

According to the FDA website on product recalls, there were 26 cosmetic/personal care product recalls for Burkholderia cepacia complex from 1/1/2018 to the present. The average number of cosmetic/personal care product recalls is nearly in number to the average yearly number of recalls for non-sterile drug products due to Burkholderia cepacia complex contamination. Based upon the number of cosmetic/ personal care product recalls due to the presence of Burkholderia cepacia complex, it looks like this microbial contaminant is just as big a problem in the cosmetic industry as the pharmaceutical industry with non-sterile drug products.

If you look at Chapter 17 – Microbiological Methods for Cosmetics in the FDA BAM, ISO Cosmetic microbial test methods and PCPC microbial count test methods, there is no information present in how to detect the presence of Burkholderia cepacia complex in non-sterile aqueous cosmetic product formulations by using enrichment.

With the exception of USP Chapter 60 for the testing of aqueous cosmetic products that are considered to be OTC drug products, my question is why there are no cosmetic microbial test methods for the detection of Burkholderia cepacia complex in aqueous cosmetic/personal care product formulations? Why has CFSAN and ISO not followed the lead of USP in requiring the testing of aqueous cosmetic products for the presence of Burkholderia cepacia complex?

Because there are no cosmetic microbial test methods for the detection of Burkholderia cepacia complex, are people in the cosmetic industry using USP Chapter 60 or an in-house test method to detect the presence of Burkholderia cepacia complex in aqueous product formulations?

My personal opinion is that cosmetic companies should be using USP Chapter 60 or an in-house method based upon USP Chapter 60 to detect the presence of this microbial contaminant in aqueous non-OTC cosmetic product formulations in order to reduce the number of cosmetic product recalls due to the presence of Burkholderia cepacia complex.

Instead of Burkholderia cepacia complex being an objectionable microorganism in an aqueous non-sterile cosmetic or drug product, it should be added for its absence as an indicator organism.

Do other members of this forum agree with my opinions?

A1: As a member of the USP Microbiology expert committee I am pleased to see your endorsement of USP <60>.

Adding Bcc as a specified microorganism in USP <1111> as a local requirement is an option as it is a harmonized chapter. Another option would be to write a chapter on the exclusion of objectionable microorganism from non-sterile drug products.

A2: Why not also add Acinetobacter baumannii in addition to Burholderia cepacia? USP add this organism to water testing.

A3: Qualitatively, I recommend a specification that establishes detection (by enrichment) of Staph aureus, Candida albicans and any Gram negative as OOS. My bias - in investigation, I'd prefer sending a Gram neg. isolate out for ID over relying exclusively on a selective medium. I understand others may find it the medium more efficient but would hesitate establishing an industry standard that demands its use.

A4: We are using USP 60 for aqueous personal care products to detect BCC.

A5: CDC presented on the issues of BCC in cosmetics and personal care products that caused severe illness/death at AFDO’s national conference. Senior FDA microbiologists agreed with CDC.

[name redacted], thank you for your collaboration with the need for this USP addition.

As y'all may recall, a former employer, Carrington Labs, here in Dallas, had one of the first major recalls for BCC contamination in their shampoos and body washes. The purified water system allowed the proliferation of BCC due its design.

Unfortunately, I see 15 years as a long time to finally see the BCC monograph. I remember [name redacted] and I talking about this issue a few times.

A6: If you have an issue for contamination in your product of a known bacteria, it also should be part of your specification not to have it in your product.Many companies have been issued 483s for not having known contaminants on your specification requirements.David Cells Sent via the Samsung Galaxy S9, an AT&T 5G Evolution capable smartphone

Allowable sample weight/volume for testing USP 61/62 and USP 2021/2022

I am looking for more information regarding the minimum weight/volume requirement for testing cosmetics/ personal care products (USP 61/62) and dietary supplements (USP 2021/2022). The monographs list the minimum requirement as 10g/10mL. The products I am testing are routine quality checks, and not release to market. Because of the scope of the testing, is there any allowable variability in sample size (i.e. using less than 10g/10mL)? What are the risks?

A1: USP <61> states:

Amount Used for the Test Unless otherwise directed, use 10 g or 10 mL of the product to be examined taken with the precautions referred to above. For fluids or solids in aerosol form, sample 10 containers. For transdermal patches, sample 10 patches.

The amount to be tested may be reduced for active substances that will be formulated in the following conditions: the amount per dosage unit (e.g., tablet, capsule, injection) is less than or equal to 1 mg, or the amount per g or mL (for preparations not presented in dose units) is less than 1 mg. In these cases, the amount of sample to be tested is not less than the amount present in 10 dosage units or 10 g or 10 mL of the product. 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 1000 g), the amount tested shall be 1% of the batch unless a lesser amount is prescribed or justified and authorized.

For products where the total number of entities in a batch is less than 200 (e.g., samples used in clinical trials), the sample size may be reduced to two units, or one unit if the size is less than 100. Select the sample(s) at random from the bulk material or from the available containers of the preparation. To obtain the required quantity, mix the contents of a sufficient number of containers to provide the sample.

The risks are reducing the sensitivity of the test.

sample size interpretation of <61> and <610>

<61> states that you need to sample and test 10g or 10ml in a 1 in 10 dilution.

When your finished product device has a volume that is 0.1ml (or it could be even less) it is really impractical and a potential for contamination to disassemble 100 complex devices to obtain 10ml and depending on the method you could need multiple 10ml's if they are processed differently for the differing routes or specified organisms and you can't use one 10ml sample in a broth/diluent to cover all testing.

<610> does state that appropriate sample quantity should be based on general test chapters e.g <61> and <62>. But, under sample size determination it says:

For each microbiological test, sample 10 drug product containers or units or a number of units that can provide a minimum of 1 gram of product that are representative of the batch.

Can i use or interpret <610> to allow me to just sample and pool 10 filled units (total 1ml) for testing (may be several 1 ml's due to different routes). I would do a 1 in 9ml dilution but then my method going forward could be a result per unit or if pour plates per tenth of a unit.

A new customer is trying to state that <610> allows for 10 units to be tested with a total of 1 ml and are not happy that we are suggesting that they need to use hundreds of units to have a compliant test.

Are there any examples or justifications that can be used for testing less that the 10g/ml and using 10 units/devices?

A1: USP 610 applies to orally inhaled and nasal drug products (OINDP). Is that the case here?

A2: Yes it is a single dose spray spray.

A3: Here is an older, but still relevant, article by Don Singer who contributes to this forum. I'm sure he could also help clarify any questions you may have. I hope this helps.

https://www.americanpharmaceuticalreview.com/Featured-Articles/112360-New-USP-Microbiology-Sampling-Chapter-Reflects-Global-Collaboration/.

AET test for reconstituted products

Based on USP chapter (51) : Antimicrobial Effectiveness Testing; As per the procedure for performing analysis the product when inoculated with known required concentration of organisms the same is to be incubated at 22.5°C+/- 2.5°C.

Now for my query, we have a new product developed which requires reconstitution with water and to be stored at 2°C to 8°C as per the label. So in case of performing AET test, the product is to be incubated post inoculating with pathogens as per label requirement or as per the harmonized USP chapter.

A1: Quick note: USP <51> is not harmonized. And to answer your question, you incubate the inoculated material at 20-25C.

A2: [name redacted] is right that the AET is conducted with storage at 20-25 degree c and in the dark if necessary. Antimicrobial preservatives are less effective at refrigeration temperature,

You may be conducting the wrong test. If I assume that the product is not preserved and is not a multiple-use product then you would conduct a challenge test to justify the reconstitution and storage time by inoculating the reconstituted product with <100 CFU of the challenge organisms, incubating at 2-8 degree C, and conducting microbial counts at regular intervals to determine when the challenge organisms has an increase in count > 1 log.

That is a very conservative criterion currently required by the FDA.

A3: [name redacted] is correct that USP Chapter 51 is not a harmonized chapter with other pharmacopeia.

By reconstituting a product with water, you will need to use a preservative in the formulation that is 100% water soluble. Most preservatives are either not soluble or are slightly soluble in water in which a solubilizer will need to be used to get these preservatives into the water phase of the formulation. If the preservative is not solubilized into the water phase upon reconstitution, the product will be inadequately preserved.

For this reason, I tend to think that these formulations might not be preserved with preservatives and this is the justification for storing them at 2 to 8C to inhibit the growth of microorganisms

In addition, another factor is the type of water that is being used to solubilized the dry product. If it was an injection, I would suspect that you are using WFI as the reconstituting liquid.

FYI, I had worked on a non-sterile dry drug product formulation that was to be reconstituted with tap water and stored at 2 to 8C. What a nightmare!!!!. The FDA had asked about the possible growth of psycophilic organisms in the refrigerated reconstituted product. The product was never launched.

A4: However, FDA will look at a > 0.5 log increase as growth over time, and the prior time point which is acceptable would dictate the allowable storage time of the reconstituted product. You will also need to test beyond the anticipated storage time. See the following:

https://www.americanpharmaceuticalreview.com/Featured-Articles/114491-Microbiological-Quality-of-Drug-Products-after-Penetration-of-the-Container-System-for-Dose-Preparation-Prior-to-Patient-Administration/

https://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/202714Orig1s000MicroR.pdf

A5: Plzease check formulation contain any preservative or not. If not then it is a reconstitution stability study not AET, and you have to follow the storage condition as mention in label claim. also the acceptance criteria is different.

A6: We need more information to offer an opinion.

I did work with the antibiotic Cefixime for Oral Suspension which is a powder that contained Sodium Benzoate used to treat children for ear infections. The product was reconstituted with tap water and was stored at room or refrigeration temperature for up to 14 days. We demonstrated that it passed the <51> AET during the expiration dating.

A7: The product is non sterile liquid containing Sodium Benzoate. The product is to be reconstituted with water, post which has to be stored at 2°C to 8°C for multiple usage. This information is adequate or please post some more queries.

A8: With regards to AET in US and EP, does anyone know why have they never been harmonised and why is there differing criteria. What are the scientific reasons behind being different?

A9: I believe the USP <51> rationale for incubating the inoculated product at 20-25C is simulate a worst-case storage condition; even though the product's insert states to keep it refrigerated (2-8C).

A10: The design of the test differs in the testing intervals for different dosage forms, the acceptance criteria and the regulatory expectations.

In addition, the Ph. Eur. limits E. coli as a challenge organism for oral liquid products.

I recommended you read the article co-authored by Cherryl Patco (Moser) for the details:

AAPS PharmSciTech <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3066379/#>. 2011 Mar; 12(1): 222–226. Published online 2011 Jan 8. doi: 10.1208/s12249-010-9575-9 <https://dx.doi.org/10.1208%2Fs12249-010-9575-9>

Comparison of Compendial Antimicrobial Effectiveness Tests: A Review

A11: If the product has Sodium benzoate as the preservative, I’m assuming that you must have Citric acid or something similar to cause the disassociation of Sodium benzoate into Benzoic acid when the formulation is reconstituted because Sodium benzoate is not biologically active unless it is in the disassociate form by lowering the pH.

What is the true reason as to why you store the reconstituted product at 2 to 8C? Do you have challenge data that shows otherwise that the reconstituted product is not adequately preserved if stored at room temperature or is there a chemical reason as to why to store at 2 to 8C to prevent degradation of an ingredient in the formulation? What type of water is being used to reconstituted the non-sterile product formulation? If the product is being reconstituted at home, I suspect that tap water is being used. Please confirm.

A12: Sorry 20-25 degree C is not a worse case as antimicrobial preservatives are less effective at 4-8 degree C.

A13: The product does not have Citrix acid. The reason behind the low temperature incubation is the product is light sensitive and formulation has three sugars namely Sucrose, Mannitol and Tutti Frutti. Water used is PW meeting the USP requirements.

A14: What is the pH the reconstituted product formulation? Sodium benzoate needs to be disassociated to be biologically active as a preservative. If your reconstituted product is sensitive to light, why are you not using an amber glass bottle to stored the reconstituted product to prevent light penetration in which you can store the material at room temperature?

A15: Many thanks for the suggestion...will convey the same to our Formulation development scientists. But the question remains as to study at which temperature as this is going to be filed in US.

A16: The more details for this are as follows:

1) The product is a sachet containing powder and stored at 25c. This is going to be distributed to patients.
2) Post reconstituted with PW, the product would.be exhausted within 7 days.....as it is 175 ml reconstituted sachet
3) The product does not have Citric acid
4) The product is to be stored at 2°C to 8°C as it is degrading in aqueous form at room temperature.

Kindly suggest if AET is still required and if so....at what temperature the product is to stored post mixing with Organisms.

A17: Yes, as a preserved, aqueous, multiple-use product it must pass a AET at zerotime and 7 days.

Whether the storage would be at 2-8 or 20-25 degree C is debatable. The AET as described in USP <51> specifies a 20-25 degree C storage irrespective of the storage temperature.

Change in fill weight (non-sterile product)

I have an inherited method for a non-sterile nasal dry powder.

10 units are pooled and tested with results expressed per unit. Criteria from 1111 applied per unit rather than 1g.

Each unit contains 25mg of product powder (APi and excipients) so when 10 are pooled only 250mg is tested. The 10 units are added to 100ml of broth and processed in the test routes.

My scenario is that we are looking to increase the dosage strength without impacting formulation. The suggestion is that more product powder will just be put in the device so that the patient would then get the higher amount of API. Each unit will increase from 25mg to 35mg and therefore 250mg to 350mg in the 100ml broth.

With this proposed change is there a justification that the MLT suitability is not required to be repeated using the new higher dose amounts?

TOC limit in PW

i’m just looking for detail on why the TOC level of 500ppbC has been assigned. Is it a comparable limit to the oxidisable substance test? What impact is there from having TOC above this e.g 1000ppbC?

A1: The determination of the TOC limit was made in ~1995-ish when the TOC method was being developed. There are article's in USP's PF that I could dig out - maybe. The limit was based on work that the Water Quality Committee of the PMA (now PhRMA) developed. [Note: In the late 80s and early 90s, PMA and USP collaborated to update the water testing. PMA had the resources and expertise, and USP had the interest.] PMA surveyed industry about their "TOC values" in their water systems - details I do not know - other readers may. Then the data was turned over USP, and they analyzed the data and determined a reasonable limit of 0.50 mg C/L, i.e., industry could achieve that TOC level. "500 ppb" was not determined to be the "safe" amount of allowable TOC. Other readers may be able to fill in blanks.

The TOC test was not, and is not, comparable to the OS test in any chemical or mathematical way. The OS test is purely optical (by eye, not spectrophotometer). There was no chemical or numerical validation that the TOC was equal or superior to OS. But it was well-established that a TOC test (compared to OS) is more sensitive, quantitative, could be used in the lab or on-line, could be for process control as well as QC, and is inclusive (detects all organics found in water systems). The OS test fails in each of these regards.

Like any TOC (or OS) test, they tell you nothing about the specific impurities. It is the chemical makeup of the impurities that may have a health impact, and the TOC test tell you nothing about the harmful/harmless organics. Same for conductivity. The purpose of the TOC and conductivity tests is to provide evidence that the water system is under chemical impurity control. You have a certain amount of inorganic and organic impurities in your incoming water, the purification system removes them by carbon, RO, filtering, EDI, distillation, etc..., and you measure throughout the water system to assure each process step is doing its job, i.e., reducing chemical impurities. The "low limits" for the cond and TOC tests provide evidence that the water system is working properly, at least from a chemical perspective. They assure that impurities in the high TOC/cond incoming water are reduced by a few log by the water system.

Chlorhexidine gluconate Oral Rinse Product Recall due to Burkholderia Contamination

On the FDA website today, there was the presence of a voluntary product recall for several lots of a Chlorhexidine gluconate Oral Rinse USP, 0.12% for the possible presence of Burkholderia lata contamination. Burkholderia lata is a member of Burkholderia cepacia complex. This recall product is also an alcohol-free product formulation that does not have a preservative system.

This antiseptic product recall is interesting for several things. Was USP Chapter 60 had been used in conducting microbial limits release testing of the recall batches of this product formulation? Due to the expiration dates of the recalled batches, it could be very possible that Quality Control microbial release testing of these recalled batches had been conducted on them before USP Chapter 60 became effective in November 2019. Furthermore, you cannot assume that Chlorhexidine gluconate would have antimicrobial activity against Burkholderia cepacia complex because it has been reported in the literature that Burkholderia cepacia complex strains are somewhat resistant to the antimicrobial activity of Chlorhexidine gluconate (Reference: Kim, J.M., et al. Survival and susceptibility of Burkholderia cepacia complex in chlorhexidine gluconate and benzalkonium chloride. J. of Ind. Micro. and Biotechnology, 42 905-913, 2015). It would also be interesting to see the Antimicrobial Effectiveness Test results for this formulation if Burkholderia cepacia ATCC 25416 had been used as one of the microbial challenge test microorganisms. I wonder whether the USP Microbiology Committee should consider in adding Burkholderia cepacia ATCC 25416 as one of the required microbial challenge test microorganisms for non-sterile aqueous product formulations since USP Chapter 51 – Antimicrobial Effectiveness Test is not harmonized with any of the other pharmacopeias. It should also be noted that the active ingredient in an antiseptic product cannot be used to also preserve a formulation from microbial contamination that occurs during consumer usage. However, it is against cGMPs to use a preservative system to take care of a microbial contamination issue during manufacturing. Based upon the ingredients that are present in this recall product formulation, I

would suspect that the source of the contamination was the deionized water system that was used to provide deionized water of the formulation.

A1: [name redacted] the recall got my attention too.

Adding B. cepcia to the challenge organisms in USP <51> has been discussed. The current chapter 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."

A2: Thank you for the clarification that you can add organisms to the microbial challenge test panel.

Instead of an option, I think that it should become a requirement that Burkholderia cepacia ATCC 25416 be added as a challenge test organism for non-sterile aqueous product formulations and remove it as an option. Because it is an option in the current USP Chapter 51, I suspect that most companies are not including it as one of the microbial challenge test organisms of non-sterile aqueous product formulations

For your information, the PCPC challenge test methods of M-3, M-4, and M-7 for water miscible products has Burkholderia cepacia ATCC 25416 listed as an option for inclusion in performing a challenge test. In addition, I believe that ISO 11930 for challenge testing of cosmetics does not even list Burkholderia cepacia as an optional challenge test organism.

Because of the issues that this organism causes for products in the marketplace and is potential hazard to consumers, I think that it is time to make Burkholderia cepacia as a required challenge test organism for non-sterile aqueous product formulations. If it is not a requirement, I find that companies will only use the required number of organisms for challenge testing of non-sterile aqueous product formulations in which additional non-required test organisms are not included.

I believe that USP Chapter 60 is a start, but USP Chapter 51 needs to be revised to address Burkholderia cepacia contamination in non-sterile aqueous product formulations. If USP includes Burkholderia cepacia as a challenge test organism requirement in USP Chapter 51, I do not see how the cosmetic industry and ISO does not eventually make Burkholderia cepacia as one of the required challenge test organisms.

Bcc and overcoming preservative

Does anyone have any detail about B.cepacia being able to resist and attack Benzalkonium chloride therefore reducing the available amounts within a formulation to adequately preserve against other organisms e.g panel within AET <51>?

A1: In a book called “Cosmetic Microbiology” edited by Phil Geis that will be published in November 2020, I have a chapter in it on preservative resistance. In this chapter, I discuss the various mechanisms in how a microorganism can become resistant to the antimicrobial activity of preservatives. In the case of Benzalkonium chloride, there are no scientific literature reports of enzymatic degradation of Benzalkonium chloride to cause a lower concentration in a formulation. For microorganisms to become resistant to the antimicrobial activity of Benzalkonium chloride, it is by adaption due to outer membrane changes.

Generally, Burkholderia cepacia has somewhat higher MIC/MLC levels to the antimicrobial activity of Benzalkonium chloride. If a sufficient concentration is not used in a formulation, Benzalkonium chloride will not inhibit or kill Burkholderia cepacia.

Residual Seal Force Monitoring

Need opinion: How do people do seal force monitoring of stoppered capped vials when filling in an isolator? Do they test beginning, middle and end? If so, how are the vials removed from the isolator, without contaminating it?

Laminar Air Flow UV burning hours requirement

Hope your all doing well and safe. Here, I have a question regarding UV for Laminar Air Flow. Is must UV burning required for Laminar Air Flow. If not or yes give any guidance for better complies of Good Laboratory Practices.

A1: UV Burning hours is mandatory requirement to prove the integrity of LAF. Hence you can switch ON for a period of 30 minutes before start up of the Day.

A2: The burning hours of UV lamp in LAF is must and mandatory to determine the intensity of the UV light. While buying the UV lamp, the manufacturer will provide the certificate for the validity of UV light regarding burning hours.

A3: Instead of relying on the UV light manufacturer certification for the number of hours that a UV lamp can be used, I would instead recommend that the UV light intensity be checked on a periodic basis to ensue that the UV lamp is working correctly in the LAF. This check can be performed when the HEPA filter of the hood is certified for air flow, integrity testing and non-particulate counts. Unless you are keeping track of the number of hours that a UV light is on, I have found it to be easier to periodically check the light for UV intensity. It is well known that the amount of germicidal wavelength of a UV lamp will decrease with bulb age and the number of hours used.

A4: Yes if you are using UV in LAF, it is mandatory to record the burning hours of UV, but… UV is important but not mandatory for bio safety and LAF, as it is concern with personnel safety, But to prove the integrity of BSC and LAF, you need to prove your method of cleaning and sanitization with a good sporocidal disinfectant, with proper DET. After proving and with proper scientific justification you can remove UV lights from BSC and LAF.

A5: I do not understand your reasoning for removing the ultraviolet light from a biological safety cabinet/ laminar flow hood after validating the disinfectant procedure of the hood. Granted, the ultraviolet light should not on when performing work in the hood, but it is an added protection between uses for controlling microbial contamination even if you disinfect the hood area adequately before and after use.

Query for Chemical indicators Strip for Steam Sterilization

I want to know which chemical indicator strips should be used for visual confirmation of completion that sterilization cycle condition were met in pressure cooker type autoclave Steam Sterilizer?

Is class 5 type of Chemical indicators, applicable to use in gravity forced Autoclave / pressure cooker type Autoclave or can we use class 6 type of chemical indicator for autoclave steam sterilization method for visual confirmation of cycle?

is class 6 type of Chemical indicator applicable with Biological Indicator, also ?

Which Chemical Indicator Strip, we can use in Autoclave for different temperatures and time parameter?is any single chemical strip available which may work on different temperatures?eg., at 121C, temperature for 15 minutes . 121C temp. For 30 mon., 115C temp., 15 minutes, 121C, 30min?

Timing of Media Fills

The companies I have worked for have done media fills after shutdown.

Is it recommended to do them before or after shutdown or both? Is there any written guidance on this?

A1: After shutdown makes sense to me.

A2: I'm typically use to Media Fills being performed after the last batch prior to shutdown. The revised Annex 1 (Feb. 2020 version) provides some guidance.

Section 9.40 Process simulation tests should be performed as part of the initial validation, with at least three consecutive satisfactory simulation tests that cover all working shifts that the aseptic process may occur in, and after any significant modification to operational practices, facilities, services or equipment (e.g. modification to the HVAC system, equipment, major facility shut down, changes to process, number of shifts and numbers of personnel etc.). Normally, process simulation tests (periodic revalidation) should be repeated twice a year (approximately every six months) for each aseptic process, each filling line and each shift. Each operator should participate in at least one successful APS annually. Consideration should be given to performing an APS after the last batch prior to shut down, before long periods of inactivity or before decommissioning or relocation of a line.

A3: in my opinion if you perform them after shutdown, it would more adequately represent the true state of the facility and its cleaning practices. It also provides a piece of data to hopefully support the claim that no PM activities, etc, that may have occurred during shutdown, had an adverse impact on the aseptic integrity of the facility.

A4: The benefit of performing a media fill before shutdown covers the production from the previous media fill until shutdown, should a problem be detected. Doing them after shutdown tells you the system is running properly before restarting production.

A5: It is better option to make after shutdown. During maintenance there are some non sterile activities which can increase microbiological load of area. Because of this you can check the effectiveness of cleaning activity, fumigation acitivity. Also can check the system integrity.

A6: Does this mean one should be done before and one after shutdown? This might be the best approach with the caveat that it would reset the semi-annual clock by the shutdown interval.

A7: It would be less of a risk to perform media fills before and after. The reason you can bracket all of your fills between 2 successful media fills rather than take a risk of just doing a Media fill after a shutdown. If something were to happen to that Media fill you have put all of those fills at risk.

A8: It is my opinion that it should performed before and after shutdown!

A9: In my opinion the media fill has to be done before and after....as this will fulfill both bi annually and after (which is a worst case scenario). This will met the guideline requirement also.

A10: In my opinion the media fill has to be done before and after the shut down. But sometimes it should be hard to make at the two periods such as described above

The relevant text of the Annex manual on the subject is below.

9.40/ Process simulation tests should be performed as initial validation, generally with three consecutive satisfactory simulation tests per shift, and after any significant modification to the HVAC system, equipment, major facility shut down, process and number of shifts, etc. Normally process simulation tests (periodic revalidation) should be repeated twice a year (approximately every six months) for each aseptic process and filling line, and at least annually for each operator.
Consideration should be given to performing an APS after the last batch prior to shut down, before long periods of inactivity or before decommissioning or relocation of a line.

A11: What are we determining shutdown (i.e. weekly, for maintenance, monthly, etc.)? Do you perform SIP? Are the system validated? Those questions need to be asked first before you decide a more frequent media fill process. It is a risk based decision for business and the consumer.

A12: If you carefully read Annex 1 you will interpret it in a way that after shutdown you must do a media run, but prior to shutdown you should consider it (would be nice but not a must).

At least that is my interpretation of it. Best option is to have both but every media run has its cost.

A13: The necessity for BOTH before and after should be seriously pondered before choosing that route, as it may not be necessary and may introduce unnecessary challenges. It can be justified in a risk assessment which route is chosen. For example, if you have a sterile filtered product, you may be less concerned with proving system suitability of the facility than if you were producing a large molecule that is too large to undergo sterile filtration, where aseptic integrity of the facility is more critical in comparison.

Borewell water analysis as per EPA guideline

There is one query related to Borewell water analysis as per EPA guideline, if our water fails during testing in any parameter then what next? As this borewell water goes to pre-treatment system before entering to PW system.

A1: Fails microbiological testing?

A2: I believe that you are talking about well water that is used as the source water for a purified water system. Are you talking about micro or chemical out-of-specification test results for potable or drinking water that is being used to supply a purified water system? If you are talking about microbial test results for the well water, it needs to meet drinking water standards which may require treatment before use in the facility. In most cases, it is rare for well water to meet drinking water standards without some type of chemical treatment. I have found most people will do a chlorine treatment of this water before it is used in the facility by people and as the source water for a purified water system.

A3: Yes sir, I am talking about bore water which enters into pre-treatment system and converts to potable water (We are not using bore water as feed to PW system). After that it feeds to PW system.

My query on failure on both tests (i.e. chemical or microbial).

A4: You would test the treated potable water using EPA/WHO/national standard not the bore water.

A5: Without having knowledge of your pre-treatment process in taking bore water and converting it into potable water, bore water will often contain bacteria, iron, lead, arsenic, chromium 6, mercury, nitrates, sulfate, and radon depending upon the geology of the area and volatile organic compounds (VOCs).

If your system is not meeting the chemical requirements of potable water, the pre-treatment portion of your water system needs to be corrected to ensure that the generated potable water is meeting EPA chemical requirements. For example, a carbon filter can be used to remove VOCs and oxidizing filtration systems can be used to remove arsenic and iron.

To remove microorganisms, ultrafiltration can be used to remove 99% of bacteria, viruses, giardia, and cryptosporidium. Generally, reverse osmosis is not used on well water for the removal of organisms because of the low pressure that is normally associated with well water. In addition, the usage of injected Sodium hypochlorite around 2 ppm in a storage tank is also often used as a microbial control measure for well water.

In USP Chapter 1231, the source water for a purified water system needs to be following the chemical and microbial requirements for potable water. If the bore water after pre-treatment is not in compliance with

the chemical and microbial requirements for potable water, the pre-treatment portion of the water system needs to be corrected. It is not recommended that non-potable water be used as the source water for a purified water system because it may have an adverse effect on the chemical and microbial purity of the generated purified water.

Understanding Table -1 Air Cleanliness by Particle Concentration ISO 14644-1:2015(E)

Please help me to understand the table 1 of ISO 14644-1:2015(E) to define the limits for non Viable particle monitoring. EU GMP Annexure -1 followed worldwide has the simple understanding in its tabular presentation where limits for Grade A , B , C and D is provided in At Rest and In Operation state whereas ISO does not clarified the condition except class 9. Currently we are following the limits as per EU GMP Annexure -1 , my worry is that if I am complying to ISO too or need to stringent the limits ?

A1: The limits established in ISO 14644-1 are for the CLASSIFICATION of the room. The limits established in Annex 1 are for the routine (static and dynamic) MONITORING of the room.

In-use microbial study for a new generic DP

I would appreciate input for a new parenteral generic DP submission (ANDA);
The DP is required to further dilute for an infusion solution preparation by an end user. The reference DP labeling insert indicates that, "the prepared infusion solutions if not used immediately can be stored between 2°C and 25°C for 6 hours (including the 1 hour intravenous administration). In addition, the prepared infusion solutions physical and chemical in-use stability has been demonstrated in non-PVC bags for up to 48 hours when stored between 2°C and 8°C (36°F and 46°F)".

Now with respect to the microbial in-use stability:

* Should in-use microbial stability be performed on all three ANDA primary batches? OR the study on a Lab scale batch can be used for a submission?
* Which is an appropriate approach, doing only at 25°C after 6 hours storage Or perform separately for 2°C and 25°C?
* Should there be a separate in-use microbial stability in non-PVC bags at 2-8°C for 48 hours?

A1: You should conduct a study using representative microorganisms inoculated into the product in the IV bag to give a challenge of 100CFU/mL to support the package insert instructions for use. The FDA takes a conservative position and expects that the log increase is not greater than 0.5 logs.

A2: I can only guess what instructions for use you will propose in the regulatory submission.

I would study 6 hours at 25 degree C and 48 hours at 2-8 degree C. You could conduct the study with lower volumes in a glass container but conducting it in the IV bag would be preferred.

A3: Additional guidance may come from a 2011 FDA presentation on similar topics. Here are some take-home points:

The storage conditions [e.g.: diluent(s), storage temperature(s)] should simulate those described in the label. So, if you have a wide range (2 to 25; I would perform the studies at the extremes.

Periodic sampling times should be performed and include time points that are 2-3 times that of the requested maximum hold time.

Perform the studies in the relevant packaging, unless you can justify otherwise. So, perform the studies in the different bag material.

what are the differences for method suitability between Di

What are the differences between Microbial method suitability requirements under Nutritional and Dietary supplements chapters (2021 and 2022) and Non-sterile pharmaceutical chapters (61 and 62)?

In 2021 and 2022 specify to inoculate test article with 25-250 cfu where as 61 and 62 specify with <100 cfu.

In 2021 specifies using S.aureus, E.coli and Bs for Total count validation whereas 61 specifies S.a, P.a and B.s.

In 2021 specifies a recovery of greater than 70% where as < 61> specifies 50-200%

Just wanted to make sure if the above are correct and if there are more differences.

A1: You are correct that the suitability testing between USP chapters 61/62 and 2021/2022 are different from one another. In addition, the amount of Polysorbate and Lecithin in TSB is specified in USP 2021/2022 are specified as in comparison to 61/62. In general, most nutritional/dietary supplements are ingested in comparison to non-sterile pharmaceutical products. For this reason, the types of preservatives used are limited in aqueous nutritional/dietary supplements in comparison to aqueous pharmaceutical products. You will find that parabens, sorbic acid and benzoic acid are often used. I suspect that this is the reason that you do not see a table of preservative neutralizers in USP Chapters 2021/2022.

EM limits calculation

There are no regulatory guidelines regarding limits calculation and some approaches are discussed in the PDA TR13-Fundamentals of EM, they are:

* Cutoff Value Approach - 95th percentile value for alert
* Normal Distribution Approach - AVG+2STDEV for alert
* Nonparametric Tolerance Limit Approach - 95% (K=95) confidence that 100(P) of a population lies below the value, as determined by the limit

According to aforementioned PDA TR13 we are not encouraged to use Normal Distribution Approach since EM data is not normally distributed but skewed toward zeros, yet this is the most commonly used approach per my experience. These three methods give significantly different outcomes in numbers. And taking into consideration the skewness of the data it makes use of statistics overly complicated (for me).

Which of these approaches are most accepted by the regulatory agencies, how do you calculate EM limits?

A1: Within critical areas of the aseptic parenteral facilities and all the "0" CFU data points, many firms use Poisson or Nonparametric. Either way, you'll need to justify your levels based on history and/or your EMPQ with regards to the criticality of the EM data on your product.

A2: I've been involved extensively with this subject for many years. My perspective has evolved over this time, becoming more pragmatic. There's actually a very simple approach... First, keep your Action Levels at (or slightly below) compendial/regulatory limits. If your facility is historically clean, you'll very rarely reach these. For your Alert Levels, use the cut-off value approach. The TR13 cut-off values are only a guideline. It specifically states: "Other percentiles may be used in establishing levels". Again, if your facility is historically clean, do yourself a favor and use something like a 99% cut-off value for your Alert Levels. You'll find that these are FAR below your Action Levels, and you won't be chasing ghosts every day.

Also, as an (almost) aside... Don't send out APB's "We got an Alert Level excursion!" to multiple departments. It quickly turns into white noise to everyone. Do a simple (usually one page) evaluation within the QC Micro department, and only raise the alarm if you find an adverse trend.

A3: Based on wide experience at multiple manufacturing sites I would recommend the non-paramteric tolerance limit approach.

A4: A little off topic but still on EM. How common or necessary is the use of a Positive Hole Correction Table (based on JM Macher's 1989 paper, Positive-hole correction of multiple-jet impactors for collecting viable microorganisms) for recording bioburden using active air sampling EM. We are a non-sterile household product/cosmetic/OTC contract manufacturing facility and our counts vary significantly through-out the facility (mostly non-zero counts). We've gone through several different types of samplers in the last few years and the newest one came with an PHC Table, which, in theory, could give you a more accurate viable measurement per air volume.

A5: I've seen both. The Feller correction table for our 300-hole MAS head becomes useful around 17 colonies. This is a theoretical number and some companies just use what is "actual" vs what is "theoretical". The table is commonly used but also commonly...ignored :)

Residual VHP effects testing for culture media

I am preparing to evaluate environmental monitoring culture media plates (settle plates) for use in a manufacturing isolator that will be subject to a VHP cycle by conducting a growth promotion test after exposure.

My question is how long (in theory) can I wait between the cycle end and the growth promotion test? Would it be advisable to let them wait for a day?
And- can anyone recommend a reference for performing similar studies.

A1: Since the inoculation procedure is straight forward, you may want to perform the testing right after a cycle "and" a day later. You should also use the plates in the isolator for the recommended use time to ensure that H2O2 residues in the air and/or on specific surfaces will not lead to a false negative EM result. If the results are satisfactory (counts between exposed and unexposed plates are similar), you should have all eventualities covered.

A2: Are these settle plates still wrapped in their packaging, as in they're being decontaminated as part of an isolator load for use during manufacturing? I would think you'd want to conduct growth promotion immediately after your aeration phase finishes and you can remove them from the isolator. If there's a situation where VHP leeches through the plastic packaging, letting them sit for a day would not be representative of conditions at the time of use as residual peroxide could dissipate.

A3: Can you measure the concentration of VHP in the air?

A4: I have experience organizing similar studies whereby the EM media were exposed in their final packaging how they normally would be part of the validated load pattern. The media were then exposed to a double VHP cycle as "worst case" and then removed from the isolator once the PPM levels were below the safe threshold to open the isolator doors. There was no delay in completing the growth promotion as these commenced on the same day.

A5: Just a brief note to be clear, media are single packed and hanging during VHP cycle, then unpacked, used and later placed for incubation. What is logical to me is to validate your conditions of use + worst case, i.e. expose them after the VHP cycle and rest them in the isolator for some time you wish to validate (e.g. 6, 12h). After that you can do GP. Some manufacturers put sodium pyruvate in media to neutralize remaining VHP residues which should not be there when the plates are used anyway.

A6: For the heipha study, we waited 20 minutes post-VHP exposure to allow it to “dissolve” into the agar, prior to inoculation.

Terminal sterilization

Please advise me as per the biological indicators used for product terminal sterilization in combination to autoclave temperature/time chosen parameters.

USP & EP Monograph for Oxygen and Carbon Dioxide

The compendia USP & EP) monograph for Oxygen & Carbon Dioxide compressed gases does not list microbial requirement. Is any guidance suggest microbial enumeration requirement for Oxygen & Carbon Dioxide compressed gases that uses in the biological therapeutic drug products.

A1: You are correct that there is no USP or EP microbial requirement for Oxygen and compressed Carbon dioxide gases. In general, I do find that 0.22-micron filters are present in these systems to control the microbial content of the gas and people will periodically check the gas for the presence of microorganisms. As far as microbial requirements, I would use microbial requirements that are found for the different types of air classifications depending upon if the product is sterile or non-sterile.

A2: There is ISO 8573 part 7.

A3: Depending on where you look, EU Annex 1 (2020 draft) & Japanese GMP simply state that the compressed gas must be "of appropriate microbiological purity". USP <1116> states that compressed gas should be included in your viable EM.

FDA Aseptic Guidance document offers a bit more clarity and states the following " A compressed gas should be of appropriate purity (e.g., free from oil) and its microbiological and particle quality after filtration should be equal to or better than that of the air in the environment into which the gas is introduced."

If you are using the compressed gas in controlled areas (e.g. Grade A, B, C or D), those gases would be the same viable action limits as those for the room active air samples (<1, 10, 100, 200 CFU respectively).

A4: The microbial requirements are mentioned in ISPE. Please go through the guideline. Futher the limits are based upon the classification of the area where samples are collected.

A5: Read PDA TR13.

Disinfection Validation

A Query for Disinfection validation<1072>:

1_ Penicillium chrysogenum’s requirement for a validation study of
non-sterile facility.

2 _Use of different ATCC number organism effect on recovery e.g Escherichia
coliATCC11229 vs ATTCC 8739, USP <1072> vs <61>.

3 _What signify having use of a different ATCC number in <1072> by USP?

A1: Bear in mind that USP <1072> is a general informational chapter not regulatory guidance or GMP requirements. The chapter is mainly directed to aseptic processing.

A2: In the latest version that I have of USP 1072, ATCC 11709 is no longer called *Penicillium chrysogenium*. Its name has been changed to *Penicillium rubens*. As far as *Escherichia coli* ATCC 11229, it is the *E. coli* strain that is normally used in an AOAC disinfectant test method for determining the bacterial resistance of disinfectants. *Escherichia coli* ATCC 8739 is mostly used in determining the antimicrobial activity of preservatives and a Quality Control strain in USP 62. In AOAC 960.09-Nonhalidide-Based Food Contact Sanitizers; Germicidal and Detergent Sanitizing Action of Disinfectants, *Escherichia coli* ATCC 11229 is listed as one of the test organisms.

However, I believe that this method is used to determine the minimum acceptable concentration of a chemical that can be used on pre-cleaned, nonporous, food contact surfaces. The AOAC 960.09 method is especially appropriate for food contact sanitizers that are intended to be diluted and that do not contain halogens. However, I do find it strange that the USP would include a sanitizer test organism for food contact surfaces in this informational chapter. Instead, I think that it would be wiser to list the test organisms that are present in either AOAC 961.02-Germicidal Spray Products as Disinfectants or in the AOAC Use-dilution Methods (955.14, 955.15 and 964.02) that are used to test sanitizers/disinfectants on hard, non-porous surfaces which would be more appropriate for a cosmetic/pharmaceutical facility.

The only other reason that I could think as to why the USP Committee that is responsible for this chapter had included *Escherichia coli* ATCC 11229 as a possible test organism because *Escherichia coli* is present as a test organism in USP Chapters 51 and 62 . However, it would be very unusual to detect the presence of *Escherichia coli* on facility and manufacturing equipment surfaces for disinfection in a cosmetic/pharmaceutical facility unless there was a major hygienic failure in the facility. I do know of one case in which *Escherichia coli* was present due to a sewage backed up into a pharmaceutical manufacturing area in which there was not a suitable air-break between the sewage system and manufacturing drainage system of the facility.

In Process Bioburden volumes

I was wondering if there was any guidance on reducing the volume used to test in-process bulk product for bioburden (for a sterile finished product) based on the batch size. I understand USP <61> gives guidance on reduced volumes based on certain criteria, but this is mainly for api/excipient testing or non-sterile finished products, the chapter doesn't mention in-process testing. I am used to seeing 100 mL as the standard for in-process testing, and in some instances 10 mL, but for very small batches where the final bulk volume might be less than 1L for example, would it be justifiable to test an even smaller volume than 10 mL for in-process bioburden? Would it be appropriate to use something like a 1% approach?

A1: There is a 1% rule for ATMPs / cell therapy products but this is for final product sterility testing at release. Usually for these products, such as CAR-T, there is plenty of upstream material for micro testing (spent media, etc.).

That being said, you can justify using smaller volumes based on a limited batch size, as long as you justify the sample volume used will give you an adequate response in the event organisms are present.

A2: BPOG published a paper regarding this subject about 5 years ago. It's also in the PDA Journal of Pharmaceutical Science and Technology, in May/June 2015.

Search this string: Microbial Monitoring For Biological Drug Substance Manufacturing: An Industry Perspective.

Annual Re-EMPQ?

I am looking for opinions on the need to do an annual EMPQ re-qualification of an area even if there weren’t any changes to the area. I’ve seen it only done if major changes are made and I believe also on a routine annual basis (abbreviated testing).

A1: As a general rule, no, an annual EMPQ is not routinely performed. If you are not performing data analysis / trending on a regular basis, an annual trend analysis and Alert/Action level review would be expected. That said, I can imagine scenarios where you might do an annual comprehensive monitoring where you have a much-reduced number of sites or frequency of monitoring that has been put into place over time.

A2: I do not get the EMPQ terminology. Perhaps an imprecise use of language.

In critical aseptic processing areas EM is conducted each shift. You would generate an annual report looking for adverse trends, review the cleanroom certification, the emergence of a new microflora, and suitability of the current plan sampling plan.

A3: In my opinion, EMPQ only needs to be performed when an area is changed or when a new building is built. Routine EM, trending of the data, and a sound cleaning and disinfection program should be sufficient to show the areas are in control.

What is done on an annual or a bi-annual basis is qualifying the HEPA filters.

A4: There is no guidelines mentioned about annual requalification for EMPQ but you can perform to reevaluate your sampling locations by considering obtained trend data. Based on the obtained requalification data hygienic status of the clean room shall be assessed and can be reevaluated.

Analysis of the Microbial Risks Associated with COVID-19 Vaccine Administration

I did this simple analysis of microbial risk and product conservation of the Pfizer/BioNtech COVID-19 vaccine.

Distribution and Administration of the Pfizer/BioNTech COVID-19 Vaccine

Distribution or Administration Step

Microbial Contamination Risk

Product Conservation

Shipment in customized containers of 195 or 975 5-dose vials at -80 to -60°
C.

Loss of container-closure integrity during shipment. Stopper formulation must be designed for -80°C storage, i.e., low compression set.

Shipments of 975 or 4,875 doses may not match the typical number of individuals being vaccinated at a single site. This will be a problem in rural areas and smaller long care–assisted living faculties.

The 195-vial pack may take 3 hours to thaw at 2 to 8°C or 30 minutes at 25°C.

None, if CCI was maintained.

DET by manufacturer

What regulations and extent of testing do the manufacturers follow in Europe for their development? For the routine manufacture and release do they then do some reduced testing and what are the requirements for this?

Settling Plate Monitoring in Clean rooms

I have an inquiry regarding requirement of routine Settle plate monitoring in Cleanrooms (especially at Grade C and Grade D). Per the current version of EU Annex 1, Manufacture of Sterile Products and FDA's guidance for Industry "Sterile Drug Products", there are specifications per Grades for Settle plates. I know in EU Annex, there was a notation says it is only applicable for Critical monitoring, but it was removed in the current version.

I wonder if performing Settle plates on a routine basis in the cleaning is required as there are specifications and how other companies are monitoring their cleanrooms.

Thank for reading this. It would be appreciated if you could share any of your experience with auditors, or your current practices at your company.

A1: We perform settle plate monitoring in our Grade A isolator only. We are still far from being audited by the EU so I cannot tell you their take on it. However, in our case the isolator is the only place that the product (post sterile filtration) is exposed for liquid vial filling. We sample from the beginning of the process (vials entering the Grade A) through to the cap/crimp area as that is when the product is considered in its sterile container.

Covid-19 Vaccine

Do you have any experience in the bacterial endotoxin assay using cartridges (p.ej. Charles Rivers PTS), applied to this vaccine?

A1: You may need to break the lipid nanoparticles to conduct the test. I would not anticipate a problem but they would need to calculate the MVD meet the USP <85> method suitability requirements of enhancement/inhibition and linearity.