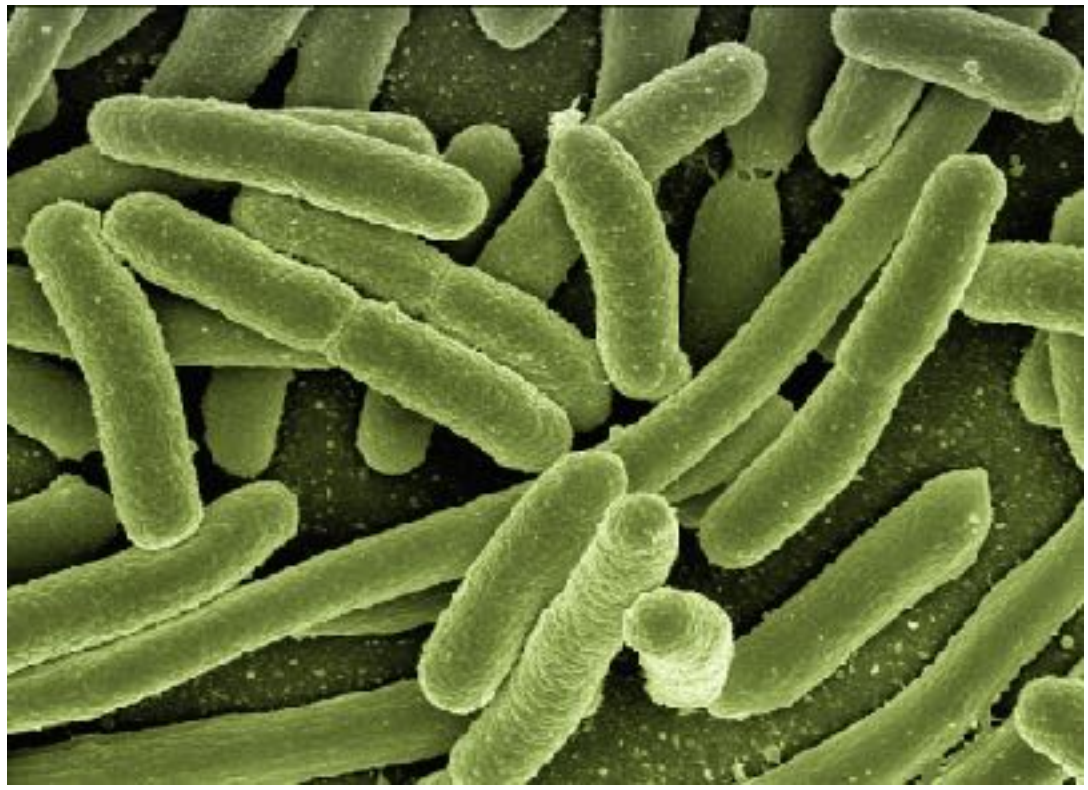
PMFList Yearbook

2022



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 2022. 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 2023 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.

Personnel and Grade D Monitoring

I have questions regarding Environmental monitoring, when the aseptic liquid filling is a closed Isolator System. One of our client who will be manufacturing aseptic DP (liquid in vial) for us, doesn't have Grade B in the controlled area where the filling Isolator is. They explain to us that their Isolator is closed system and therefore there is no Grade B (Isolator is in Grade C area) hence no personnel monitoring required. Is this as per aseptic processing cGMP? Also, their Grade D area is monitored only "At Rest" condition not > monitored at "In Operation" condition. Is this as per aseptic processing cGMP?

A1: The short answer to both of your questions is yes, this is likely to meet cGMP. The design, installation and usage of the specific isolator will determine the classification needs/requirements of the surrounding area. In my experience it has been very unusual to build Grade B around an isolator unless a specific application required it. Personnel monitoring in the surrounding area would also be unusual unless there is some particular risk to the product or process. Most isolators have gloves and they are most appropriately considered to be part of the interior surface monitoring program, not personnel monitoring. There is generally broad latitude for monitoring a Grade D environment. You should review the documentation for establishing the EM program to ensure it includes appropriate justification for the monitoring sites, frequency and conditions.

A2: Personnel monitoring for gown and gloves are required if the people are aseptically gowned i.e Grade B environment.

Not required or recommended by any regulatory body for grade C environment.
Also limits for gloves and gowns are defined for grade C environment.

Swab test recovery study

Can anybody explain how swab recovery study is done / performed?

A1: A swab recovery test is described in the 17th Edition of the APHA Standard Methods in the Examination of Dairy Products and has a recovery rate from 30 to 60% depending on the challenge organism. As a Standard Method, why would you validate the recovery?

A2: We have a validation study for our swabs that are presence/absence. The recoveries of swabs in publications has been anywhere from 25-70% so it's a wide range. PDA has papers that talk about other swab recoveries. Email me directly for a copy of the swab validation.

A3: Flocked swabs have a recovery around 60-70%. You can find recovery validation studies performed by pharma companies in internet.

Probiotics in cleaners

I read a lot of cleaning products that contain 'Probiotics' as enzymatic cleaners. If adding active cultures [initially as spore bundles] but eventually will have to grow and get active to produce respective enzymes on sites to clean the surfaces by breakdown of organic soils from surfaces, not by 'killing' harmful bacteria but by replacing themselves as 'good' bacteria. My question is , where does the surface 'hygiene' aspect come into play! What is the bioburden of these beneficial bacteria [mostly Bacillus] allowed on surfaces? Are there any regulations? How does the regulatory body view and approve such products? If they are used in industrial cleaning set up and manufacturing sites, invariably they will overwhelm and create other issues. Can the experts help in explaining how 'Probiotics' work in industrial cleaning.

A1: Bacterial enzymes are already in some detergents and cleaning agents. These are produced by bacterial fermentation and purified so it is not necessary to add live organisms to a cleaning process.

A2: I know urified Enzymes are marketed, but live 'Probiotic, bacterial spores in a formulation to be sprayed on hard surfaces🤔 and expecting it to dominate on surface as ‘beneficial' population than other 'harmful' non-beneficial organism.

A3: It's not the pure enzyme claims, but live 'Probiotics' concept making analogy to replacing gut bacteria on replacing microflora on hard surfaces!!! That's why I wanted to know regulations for spraying bacterial spores.

CRITERIA FOR MICROBIOLOGICAL METHOD VALIDATION

I want to khow what are the acceptance criteria for reproducibility and repeatability in total count method validation?.

What is the analytical reference of these criteria?

Microbial analysis of acid or base

Dear all , what is your opinion on performing the microbial analysis (Bioburden, BET) of acid or base (specially strong acid or base).

A1: When it comes to acid or base we also must be open of acid content. As you told strong acid what could be water content available in particular product. If its very little you can justify the test based on water activity chapter of USP.

A2: The analysis of the acids and bases used at the level of microbial recovery, perhaps, does not have as much impact at the level of microbial recovery.

In particular, I perform the endotoxin test on acids and bases because these used, in cleaning or formulation, can alter the load of endotoxins by breaking the cell wall of microorganisms.

suitability of the test method azithromycin microbiology

I have a problem with the suitability of the test method azithromycin microbiology, Staphylococcus A, Pseudomonas and bacillus did not grow.

I have made up to 3 dilutions but I manage to recover the strains ... I also tried the filtration method and union of both.

If anyone has had this problem please share.

I fully understand that azithromycin blocks certain cellular functions in bacteria, and if anyone knows how to inhibit please share.

Note: Candida and aspergillus grew very well (of course they are fungi).

A1: Perhaps this paper on Clarithromycin can help. Just a 1g/100mL Lecithin seemed to work.

A2: Have you tried adding Magnesium chloride as an ingredient to your diluent to neutralize the antibacterial activity of azithromycin.? It is well known that Aluminum and Magnesium salts in antacids will neutralize the antimicrobial activity of macrolide antibiotics such as azithromycin.

BCC for Non-Aqueous Products

We've been getting feedback from FDA reviewers with respect to microbiological specifications for newly submitted NDA/ANDA filings with respect to BCC. It was my understanding that BCC is a concern for aqueous products and we have been including BCC testing for our products containing water.

However, most recently we've received a request from FDA to add BCC testing for a product that doesn't have any water in its formulation at all, and none of the excipients are hydrous. This is for a petroleum based topical ointment.

I suppose one could argue water is used in equipment cleaning for the product, but by that rationale BCC testing would be required for all products.

What are your thoughts on this?

A1: We asked this question many times of the FDA/USP and were told “it’s for aqueous, non-sterile products only”. This might have to do with the latest recall of an ointment that was used for burn victims who would have a disrupted epidermis, possibly allowing a contaminant to create an opportunistic infection. The guidance was before this ointment (and a room spray) was recalled with a Ralstonia/ Burkholderia contamination. Perhaps the thinking is changing? Or is it just because the ointment is used on high-risk patients like burn victims?

A2: It’s not necessary and residual purified water rinse (via equipment cleaning) would be a weak argument.

A3: The FDA emphasizes a division into aqueous and non-aqueous non-sterile drug products based if water is an ingredient when the critical parameter is water activity. For example, no microorganism will grow at a water activity less than 0.6. See USP <1112> for details.

With a pharmaceutical ingredient with a manufacturing process that lessens the risk of microbial and a low water activity that cannot support microbial growth like petroleum would not require screening for Bcc.

A4: BCC contamination of nonsterile products have included many different types of formulations, and FDA is coming down hard on companies to ensure they have addressed the potential for this organism to be present. Much of my work these days are investigating and remediating BCC contamination across many different types of dosage forms and product configurations.

Although a product such as yours may not be expected to allow the proliferation of BCC (i.e., low water activity), its presence at low levels, even in a topical formulation, may still warrant testing.

A5: Also, for petroleum based product one should have solvent rinse or adequate drying to ensure there is no moisture left. So, you could show that the cleaning and sanitization is adequate for anhydrous product and there is no risk for BCC.

A6: I would like to provide some clarification for this posting. First of all, the recall of the Clobetasol propionate ointment was due to the presence of Ralsontii picketti contamination. This formulation is not 100% non-aqueous. If you look at the ingredient listing of this formulation, purified water is present in the formulation. It is highly likely that the source of this Ralstonia contamination in the product is the water from the purified water system. This recall ointment product is not 100% non-aqueous.

As far as the FDA making it a requirement that non-aqueous product formulation should be tested for the presence of Bcc, I do not agree with it unless there is the possibility that an ingredient in the formulation may be susceptible for the presence of Burkholderia cepacia complex contamination.

However, there are times that you cannot fight city hall if the FDA is making it a requirement that Bcc should not be present in non-aqueous product formulations. Like Mike, I have also come across this issue about the FDA making it a requirement that non-aqueous product formulations should be tested for the

presence of Burkholderia cepacia contamination no matter what the USP Microbiology Committee states in USP Chapter 60.

A7: More detailed information can be found in the articles below (along with plenty of references). My work predates <1112> and <922>.

Friedel R.R. "The Application of Water Activity (aw) Measurement to the Microbiological Attributes Testing of Raw Materials Used in the Manufacture of Non-Sterile Pharmaceutical Products," Pharmacopeial Forum, Vol. 25, No. 5, pp. 8974-8981, 1999.

Friedel R.R. and A.M. Cundell. "The Application of Water Activity Measurement to the Microbiological Attributes Testing of Nonsterile Over-the-Counter Drug Products," Pharmacopeial Forum, Vol. 24, No. 2, pp. 6087-6090, 1998.

A8: Thanks for the feedback so far. To give a little more information about my situation, the water activity for this ointment measures around 0.2 Aw and no, none of the raw materials making the product are naturally derived, hydrous, or have any other red flag for higher susceptibility to BCC contamination.

A9: If you want to ensure that no petroleum ointment residues are no longer present in manufacturing equipment, you need to be careful in what type of petroleum solvent that is selected for use. For example, petroleum is soluble in glycerin and propylene glycol, but it is also soluble in isopropyl alcohol. I would think that it would be more appropriate to use isopropyl alcohol as a final cleaning rinse for removing petroleum ointment residues from manufacturing equipment before conducting equipment sanitization. Any remaining isopropyl alcohol residues from cleaning would evaporate from the manufacturing equipment. However, there is still the flammability issue of using isopropyl alcohol in a manufacturing environment.. If you use glycerin or propylene glycol, you will still have to use a water rinse such as purified water to remove any residues of these 2 solvents. In addition, I would recommend staying away from using strong petroleum solvents such as acetone, methyl ethyl ketone, toluene, and trichloroethylene for usage in cleaning manufacturing equipment for obvious reasons.

A10: The FDA's emphasis on Bcc (and other objectionable organism) control in topical products likely stems from the contamination of ultrasound gels and other aqueous topical products over the past two decades (see the links below). I think there's a real concern that Bcc, primarily a lung pathogen, should also be absent from topical products, even if the water activity does not allow for Bcc proliferation. In other words, topicals products are expected to demonstrate an absence of Bcc just as they are expected to show an absence of S. aureus or P. aeruginosa. In my opinion, Bcc is problematic because it's capable of growth in weakly preserved aqueous products to levels capable of causing serious infections, particularly in immunocompromised patients. I'm unaware of any reports of Bcc infections resulting from the use of non-aqueous products. However I know that certain non-aqueous inhalation products are subjected to Bcc release testing based upon the intended patient population. It all comes down to the risk of <100 CFU/g (the TAMC limit for most topicals) is and whether that warrants Bcc testing of all topical products. Upstream controls demonstrating the absence of Bcc in the manufacturing process may allow for a waiver of finished product testing.

A11: For many years, people and publications have indicated that if a product formulation has a water activity level of 0.6 or less that it does not need to be tested for microbial content. With the FDA demanding that Burkholderia cepacia complex testing be added as a specification for non-aqueous products that have a low water activity level, I'm wondering if this new direction is coming somewhat from a FDA draft guidance document called Microbiological Quality Considerations in Non-sterile Drug Manufacturing that had been published in September 2021.

In lines 214 to 217 of this draft document, it reads as follows:

However, it should be noted that although microorganisms that are present in a non-sterile drug product with low water activity will not proliferate, they can persist in non-aqueous liquids and dry products throughout the shelf life of the product.

In lines 220 to 222 of this draft document, it reads as follows:

Consequently, it is important to provide for appropriate microbiological control of the components (e.g., excipients and APIs) of non-sterile drug products, even if the components possess a low water activity.

Based upon these 2 sentences that are present in this draft guidance, it seems that FDA has indicated that they also have a concern about the survivability of organisms in non-aqueous and dry products that have a low water activity level besides of the possibility of having microbial proliferation in aqueous products with high water activity levels.

Survivability of organisms in products with low water activity levels should also be a concern from my perspective if there is the possibility of them being applied to the skin that has been injured. Furthermore, I do not think that water activity should be used as a sole criteria in determining whether a product needs to be tested or not tested for microbial content. A robust risk assessment needs to be performed in which other factors (e.g. pH of the product, presence of other hostile raw ingredients, manufacturing conditions, preservative challenge test data, etc.) are evaluated to determine whether a product should or should not be tested for microbial content.

A12: I have conveyed to some of my clients that although their non-sterile formulation may prevent the growth of organisms, the organism may still persist over time. The FDA draft guidance supports this position.

A13: As an early proponent of the application of water activity to non-sterile products as with the concept of hurdle technology from the food industry I have emphasized that a risk assessment should include microbial quality of the pharmaceutical ingredients, manufacturing processes, the formulation, and other physicochemical parameters imminical to microorganisms. With multiple-use drug products we would need to demonstrate using USP <51> that the product is either self-preserving or if containing an antimicrobial preservative passes the test. Bcc may be used as a challenge organism, when justified.

The FDA need to sharpen their recent Guidance for Industry.

A14: We should return to Robert's original question whether you would screen for Bcc for all topical products or just for aqueous products applied to broken skin, i.e., in terms of Bcc growth >0.9 water activity. If you do that you do not have a risk based approach to manufacturing and testing. I would recommend by USP <51> demonstrate that Bcc does not survive in an ointment and justify to the FDA for having no Bcc screening.

I did write an article on a related issue. Cundell, T. Is *Bacillus subtilis* Objectionable in an Oral Liquid Solution Amer. Pharm. Rev. 23 (2): 44-47 2020

A15: There is also the possibility the FDA reviewer is mistaken. The reviewer referred to this product as a "non-sterile aqueous drug product" in the correspondence text. I've seen reviewer errors before. For example, I've been asked by an FDA reviewer to provide missing S. aureus and P. aeruginosa USP <60> suitability results for a submitted BCC test method on a different application.

A16: As far as the usage of hurdle technology, I have heard the usage of this term for both food manufacturing and cosmetic product preservation.

It is my understanding that hurdle technology in the food industry is actually a method for ensuring that microbial pathogens in food products can be eliminated or controlled by making food safe for consumption. For foods, this method generally includes the following steps: cleaning of manufacturing equipment, sanitization of manufacturing equipment, no cross contamination, training, environmental sampling and the usage of low temperatures. From my perspective, I have never seen the usage of water activity as part food hurdle technology.

For product preservation, the usage of hurdle technology was used to determine whether to include or not to include a preservative system in a product formulation to prevent microbial proliferation during

consumer use. The usage of water activity was one of the steps. I believe that Don Orth had several publications on this topic.

I just have an issue in using water activity of a non-sterile product formulation or a raw ingredient as a sole criteria in determining whether or not to conduct microbial content testing for a sample especially in light that microorganisms are able to survive in a product without proliferation. In thinking about it, I had performed sterility testing in the past on sterile non-aqueous ophthalmic ointments that had a water activity below 0.5. In playing devil's advocate, why is it O.K. to perform sterility testing on a sterile ophthalmic ointment and not to conduct microbial content testing on a non-aqueous non-sterile ointment in which both formulations have a water activity level below 0.5? I know that I'm comparing apples to oranges when it comes to sterile and non-sterile formultions, but it is still about the survivability of microorganisms in a non-aqueous product formulation whether they are either sterile or nonsterile.

As far as the recent draft guidance document, I do understand the reasoning behind FDA statements about using water activity for adding or not adding microbial test specifications for a finished product or raw material.

In light of FDA demanding the placement of microbial test specification for non-aqueous non-sterile product formulations and the recent published FDA draft concerning the manufacturing of non-sterile products, it might be time for a new guidance document to explain how to evaluate whether as formulation or raw ingredient that needs to be tested or not for microbial content like ISO 29621 and not just rely on water activity as a criteria. Instead, this proposed document should explain that the following risk assessment aspects that will need to be evaluated for determining whether a finished product or raw ingredient needs to be tested for microbial content such as pH, presence of hostile raw ingredients in the formulation to prevent microbial growth and survivability, water activity, manufacturing conditions, presence of microbial nutrients, alcohol content, type of packaging used, etc.

A17: My lab did conduct a sterility test on a sterile ophthalmic ointment. The product was irradiated using a minimal dosage due to the packaging.

The FDA Guidance for Industry must decide whether they recommend across the board Bcc testing for topical non-sterile products or testing based on a risk assessment.

A18: Part I

“From my perspective, I have never seen the usage of water activity as part of hurdle technology.”

Friedel R.R. (1999) “The Application of Water Activity (aw) Measurement to the Microbiological Attributes Testing of Raw Materials Used in the Manufacture of Non-Sterile Pharmaceutical Products,” Pharmacopeial Forum, Vol. 25, No. 5, pp. 8974-8981.

Ref # 24: Leistner, L. (1985) “Hurdle Technology Applied to Meat Products of the Shelf Stable Product and Intermediate Moisture Food Types,” In: Simatos, D. and J.L. Multon, JL. (eds.), Properties of Water in Foods in Relation to Quality and Stability, pp. 309-329.
Ref # 25: Grijspaardt-Vink, C. (1994) “Food Preservation by Hurdle Technology,” Food Technology, V. 12, p. 28.
Re # 26: Gould, G.W. And M.V. Jones (1989) “Combination and Synergistic Effects,” In: Gould, G.W. (ed.), Mechanisms of Action of Food Preservation Procedures, pp. 401-421.

Part II:

“I just have an issue in using water activity of a non-sterile product formulation or a raw ingredient as a sole criteria in determining whether or not to conduct microbial content testing for a sample especially in light that microorganisms are able to survive in a product without proliferation.”

Friedel R.R. “The Application of Water Activity (aw) Measurement to the Microbiological Attributes Testing of Raw Materials Used in the Manufacture of Non-Sterile Pharmaceutical Products,” Pharmacopeial Forum, Vol. 25, No. 5, pp. 8974-8981, 1999.

If you go to the “Discussion and Conclusions” section on p. 8976, you will see the following statement:

“When examining the need for microbiological attributes testing of pharmaceutical raw materials, careful consideration should be given to the following factors (39, 51, 71-73)…

1. Origin of the raw material: natural or synthetic/water based
2. Type of product that uses a particular raw material and route of administration
3. Antimicrobial activity of the material
4. Presences of preservatives
5. Manufacturing process which reduce or eliminate the microbial burden
6. Historical microbial profile
7. Customer Complaint history
8. Validated microbiological testing by the supplier
9. Supplier audits

After thoroughly examining each of these factors, there my be legitimate scientific justification for the reduction or elimination of microbial attributes testing of certain raw materials (74)."

Same principles can be applied to finished products. DOES THIS NOT SERVE AS A "RISK ASSESSMENT” exercise?

A19: Granted, I will admit that the references that you had provided in your response does support the usage of water activity and for conducting a risk assessment. However, your response and provided references are ignoring the current situation in which the FDA is now requesting the addition of microbial test specifications for non-aqueous non-sterile product formulations and what is written about the survivability of organisms in these types of formulations in the recently published FDA draft guidance for the manufacturing of non-sterile product formulations.

From my perspective, it seems that the playing field has indeed changed in regard to FDA’s stance regarding the presence of Burkholderia cepacia complex in all non-sterile aqueous and non-aqueous product formulations. I can see their reasoning behind their requirement in having the absence of Burkholderia cepacia complex in all aqueous non-sterile product formulations and those non-aqueous non-sterile pulmonary product formulations.

Because of these 2 above positions by the FDA in requesting microbial test specifications and the survivability of organisms in non-aqueous product formulations, it seems that it does not matter that a conclusion from a conducted risk assessment for not including the addition of microbial test specifications for non-aqueous non-sterile product formulation can no longer be justified. I do not agree with this totally, but I need to play in FDA’s sandbox according to their rules like many other individuals and companies especially if the FDA makes a request for the addition of microbial test specifications for an ANDA and NDA product formulation. If I do not comply with their request, I do not get approval from the FDA to sell the product. It is as simple as that.

A20: I see a lot of discussion and references to the FDA draft document “Microbiological Quality Considerations in Non-Sterile Drug Manufacturing”.

Just a reminder, this is a draft that everyone had a chance to comment on prior to end of last year as per the FR publication.

I am certain many of you, as did I, commented on the lack of support for appropriate risk assessments. I’m hoping that our feedback has the opportunity to be reflected in the final version.

I particularly don’t enjoy when good science is discarded in favor of simple compliance.

A21: This is a great discussion about Bcc and comes on the heels of ANOTHER Bcc-related warning letter from the FDA (see link https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/warning-letters/sunstar-americas-inc-614058-12212021 - like I mentioned the weekly warning letters are a gift that keeps on giving!). While the Bcc issue with aqueous non-sterile products is very obvious (to most - but not all, unfortunately), I want to question the seemingly apparent viewpoint that FDA is concerned about low numbers (i.e. within TAMC specifications) of Bcc species in solid oral dosage forms, raw materials for solid oral dosage forms and even the presence of Bcc in water systems used for solid oral dosage form manufacture/cleaning. I also see there is a concern for potentially long-term survival in non-sterile finished drug products (see the link to download the FDA draft https://www.fda.gov/regulatory-information/search-fda-guidance-documents/microbiological-quality-considerations-non-sterile-drug-manufacturing).

While it is true that Bcc is a very pathogenic microorganism in certain situations, is there any clinical evidence that Bcc is pathogenic in low numbers in solid oral dosage forms? The reason I am bringing this up is that I have always felt that some common sense should come into play when discussing potentially objectionable microorganisms in drug products. For example, Bcc microorganisms can survive quite happily in our drinking water and are commonly recovered from chlorinated drinking water systems - the same drinking water that I assume patients are using when taking oral, non-sterile dosage forms. Zanetti et. al. demonstrated in 2000 in Italy that:

"High levels of B. pseudomallei were recovered (mean value=578 cfu/100 ml) in about 7% of samples, while B. cepacia was recovered in 3.5% (mean value=<1) of the samples."
Zanetti, F., De Luca, G., and Stampi, S. (2000). Recovery of Burkholderia pseudomallei and B. cepacia from drinking water. Int. J. Food Microbiol. 59, 67–72. doi: 10.1016/S0168-1605(00)00255-5

In addition, Feazel et. al showed that Bcc species form biofilms in quite a few samples taken in Denver and NYC in a study from 2009.

Feazel LM, Baumgartner LK, Peterson KL, Frank DN, Harris JK, Pace NR. Opportunistic pathogens enriched in showerhead biofilms. Proc Natl Acad Sci U S A. 2009;106(38):16393-16399. doi:10.1073/pnas.0908446106
I am quite sure there are others as well

If showering (and presumably breathing aerosolized Bcc into the lungs) and drinking water with Bcc species present are common, then why are low numbers of Bcc in low water activity (where they cannot proliferate) solid oral dosage forms objectionable?

This is more of a question to stimulate further discussion. I do feel some of the sentiment that Don expressed below:
"I do not agree with this totally, but I need to play in FDA's sandbox according to their rules like many other individuals and companies especially if the FDA makes a request for the addition of microbial test specifications for an ANDA and NDA product formulation. If I do not comply with their request, I do not get approval from the FDA to sell the product. It is as simple as that."

I'd love to hear more comments and thoughts.

A22: The number of comments on the FDA NSD Guidance for Industry was underwhelming. The FDA received 17 comments with a breakdown of 8 pharma companies, 6 industry organizations, and 3 individuals. Of the 8 manufacturers there were only 4 big pharma companies.

The USP, PDA and Pharmig commented.

Yes, if you do not like the finalized document but your company did not comment you are to blame.

A23: This has been a great discussion. Thanks to all who've contributed. I'm trying to look at this from the FDA's perspective. Bcc is viewed as an organism that:

1. Is preservative and antibiotic resistant
2. Is an opportunistic pathogen frequently associated with nosocomial infections of immunocompromised patients
3. Has a history of causing infections when present in drug products that are administered through liquid oral, inhalation, and cutaneous use.
4. Is frequent contaminant of purified water systems used to make both aqueous (Aw >0.6 as defined in USP <51>) and non-aqueous products.

As such, Bcc is likely considered by the FDA to be an objectionable organism that must be excluded not only from aqueous products, but from any product with a route of administration that could cause a Bcc infection. So just as a non-aqueous topical product would be expected to include finished product testing for S. aureus and P. aeruginosa, these products are now expected to also include a release test for Bcc.

My questions is this: is Bcc pathogenic if present in low numbers in non-aqueous product or is the pathogenicity of this organism a function of it's unique preservative resistance that allows it to survive and proliferate in weakly preserved products where other organisms fail to proliferate? I can accept any answer but the answer must be based in fact so we're not tilting at windmills. My concern is that if we know that Bcc can cause infections when administered in liquid oral products and the FDA considers Bcc objectionable in non-aqueous products, will solid orals soon be required to be Bcc free? That may sound ridiculous but the line keeps moving. As stated in the FDA 's draft guidance we need "Sound scientific rationale...to prevent objectionable microorganisms from affecting the quality of a NSD”.

Stability testing of raw materials

A new customer is asking us to perform microbiological analysis of a raw materials, used for non-sterile product, at the end of their shelf life as a routine practice.

Is this necessary for the manufacturing site, either as an initial one off assessment to show that it is still microbiological acceptable at the end of its pre-defined shelf live under normal opening/usage and storage conditions? or as an ongoing stability plan for raw materials.

Do we need to worry that acceptable low bioburden material at initial testing and release could have growth occur and then could fail specification several years later? Therefore we should be doing or consider doing stability testing of all raw materials and API’s.

A1: It depends on the physicochemical attributes of the ingredient and the contribution it makes to formulation. With a dry powder with a low water activity it would have a microbial stability and no testing on stability would be justified.

Also what is the logic of testing at the end of the shelf life. If the ingredient will support microbial growth a drug substance or excipient would be at intervals as part of the requalification of the pharmaceutical ingredient.

The request appears to be driven by a misguided compliance concern and not science.

A2: A full specification testing at the end of stability is performed to illustrate that the material was good through its expiry. Technically, the material can be used till the date of expiry or the batch be released prior to the expiry date of the material (depending on how conservative a company's position is). So, it is a requirement that the material meets microbial specification at the end of shelf life. Assuming one batch per year goes on stability and not every batch.

A3: In regards to conducting microbial testing of raw ingredients after initial microbial testing has been completed, I have found that stored raw ingredients that are either natural or contain water can become contaminated during the weigh out process for batching. In addition, I have found that it is common for raw ingredient manufacturers to include the presence of a preservative system in aqueous raw ingredients

to prevent the growth of microorganisms without conducting microbial challenge testing to verify the adequacy of the preservative system in their raw ingredient. To prevent microbial contamination of a finished product, I do recommend that these types of raw ingredients be tested periodically for microbial content during storage after they have been found to be initially acceptable.

Based upon manufacturing demands, it is highly unlikely that you would have any material left at the expiration date of the material by a raw ingredient manufacturer. If your company is having material left in the facility at the expiration date, you have bigger issues from ordering unnecessary amounts of raw materials that your company does not need.

reduce testing

We perform TAMC ,TYMC & pathogen testing (E.coli,salmonella, P.aeruginosa & Salmonella) of purified water on a daily basis.We didn't find the same pathogen & TYMC in my water system.Can we reduce pathogen testing (E.coli, salmonella, P.aeruginosa & Salmonella & TYMC) of purified water on a daily basis?

A1: This is unnecessary testing and following USP 61 and 62 chapters for water is inappropriate.

That being said, E. coli or coliform testing can be performed for incoming city water on a periodic basis.

P. aeruginosa can be tested in your purified water but there are companies that have discontinued this as well.

There is no reason to test Salmonella or S. aureus. And unless you recover yeast or mold from water (which you should not), there is no reason to test for TYMC.

The TAMC should use appropriate media and incubation parameters. e.g., R2A agar for 5 days at 30-35 deg C.

Also consider BCC testing of water samples (USP 60), especially if the water is being used to make nonsterile dosage forms, especially of aqueous nature. FDA has been asking for this more often than not.

A2: I'm assuming that you are using the methodology in USP Chapters 61 and 62 to perform microbial testing purified water samples. It is highly unlikely that Eschericha coli and Salmonella species would ever be detected in a purified water system because the nutritional aspects of purified water is too low to support the survival and growth of these organisms. Generally, organisms found in purified water systems have low nutritional requirements. Because of this, plate counts are usually performed on purified water system samples by using a low nutritional growth agar such as R2A or Plate Count Agar. In conclusion, the methods in USP Chapters 61 and 62 are inappropriate for determining the microbial content of purified water samples. Instead of reducing the frequency of microbial testing of purified water samples, I would suggest that you first re-evaluate the test methodology and specifications for your purified water samples before doing anything else.

A3: You should be following the microbiological guidance provided in USP Informational Chapter <1231> Water For Pharmaceutical Purposes.

Why are you testing for Gram-negative, USP indicator type organisms in Purified Water, not to mention Total Yeast & Mold Counts?

A4: We perform TAMC using R2A agar (30 -35 For 5 days) as per you , Coliform on M endo agar (30 35 for 48 hrs) , TYMC on Sabouraud dextrose agar (20-25 for 7 days). From Soayabean casein digest medium(30-35 for 47 hours) . after that we streak Pseudomonas Isolation agar for P.aeruginosa & Mannitol salt agar for S.aureus & Inoculate 1 ml to Rappaport Vassiliadis salmonella broth (30-35 for 24 hr) & streak on Xylose lysine deoxycholate agar for salmonella (30-35 for 48hrs).

As per your opinion I can reduce TYMC ,& Pathogen from my routine water testing & only perform TAMC by R2A agar. We have been performing water testing procedures for the last 6 years . & i never got TYMC & pathogen .So how can i justify to regulatory auditors for reduction ?

Our sanitization procedure of storage tank by thermal (90C for 1.0 hr) once a month & cleaning procedure (90 c for 30 mins) on weekly basis.

A5: It all depends on which specification you are using and what kind of products are being manufactured using the water produced from the system.

Since the water which is fed into the water system for purification must be coming from Borewell water, so as per local laws, it is mandatory to perform tests for pathogens in water particularly coliforms.

Scenario 1: You have not come across any pathogenic growth for quite a long time, say a year or two and if your water system validation is complete, then in that case you can do a risk assessment and reduce the pathogen testing at some of the user points. Still you will have to check for pathogen testing at the Borewell or raw water spource, PW Generation point, Storage tank of purified water and the return loop apart from regular testing for TBC for other points. In such a scenario, you will be taking a risk but then since your Worst case point (Return loop) is being monitored on a daily basis with pathogen testing, any deviation can be handled through proper remediation. You cannot remove pathogen testing totally as you have to comply with local guidelines and EU/US Specification requirements as well.

Scenario2: Your water system is not validated and you are still generating data. In such a case it is not advisable to remove pathogen testing. There should be sufficient data to first corroborate your findings.

In both scenarios. you can remove TYMC by having a validation to support that your testing procedure is capable of detecting TYMC in case it is observed. TYMC can be kept limited to only some of the points.

A6: You have the justification. already in records. In my opinion, Six years of testing records using the appropriate agar for water, and expert advice of Internationally recognized microbiologistin in this forum, should convince regulatory auditors.

A7: Perform a risk assessment to reduce or eliminate the extra testing, based on many of the comments you have read in this thread, and the fact that you have not detected organisms in these other tests. Michael

A8: From my perspective, I'm not sure that he has justification to reduce the frequency of testing for water test samples based upon the identification test methodology that is being used. Granted, he is using R2A for generating microbial count data on tested purified water samples. However, the identification methods that are being used on R2A recovered isolates are for the most part from USP Chapter 62 for the USP indicator organisms. They are presumptive identification methods.

With the exception in the presumptive detection of Pseudomonas aeruginosa and other fluorescent Pseudomonas species such as Ps. fluorescens and Ps.putida on Pseudomonas Isolation Agar, I do question as to whether these USP Chapter presumptive identification methods would be able to identified recovered organisms that are commonly found in purified water systems such as Burkholderia cepacia complex, Ralstonia species, Sphingomonas species and et al. If I was a regulatory inspector, this point would be an issue for me that USP presumptive identification methods are being used to confirm only the absence of the USP indicator organism such as Ps.aeruginosa, S. aureus, E. coli and Salmonella species and not the microbial isolates that are normally found to be present in a purified water system.

Instead of using the presumptive identification methods, I would like to see subcultures of R2A recovered isolates are performed in which Gram-staining and biochemical identification kits or strips are used to determine a biochemical identification of an isolate in case that 16s rRNA sequencing or MALDI-TOF are not being used to confirm that absence of non-indicator objectionable organisms in purified water samples. It is time to move into the 21st century by not relying totally on presumptive identification methods when it comes to the identification of microbial isolates.

Furthermore, I would like to know what is the current microbial testing frequency of water samples that have been taken from a purified water system. Is it daily, weekly or monthly?

A9: Storage tank & Return loop on daily basis & rest of other user points covered on weekly basis.

A10: Purified water testing frequency not mentioned any guideline USP or EP . It is mentioned to perform frequencil manner.

Based validation of the water system & number of sample points , select the testing frequency supply and return on daily/weekly and remaining sample points covered monthly once is a better approach.

Regarding specified microorganisms E.coli, salmonella, pseudomonas and staphylococcus aureus there is no requirement for purified water as per guidelines(USP/EP).

But to minimize the risk in the product it is better to perform these specified microorganisms. Most of the time purified water is used during the product process or cleaning purpose .

A11: There is no requirement of TYMC and pathogen testing in water, untill or unless your local authorities/ pharmacopeia doe not recommended it.

However if it is required, you can perform reduce testing on monthly basis for supply or return loop only on monthly basis instead of all testing point

You are already having historical data to justify the reduce testing. You must have an RA.

pseudomonas aeruginosa water

We test our pre-PW system specifically for Pseudomonas aeruginosa besides
total counts.
This is a stand alone test to be sure Pseudomonas does not enter the WFI system. Possibly it is an indicator of biofilm in the system.
I have not seen a good correlation with total counts.
Is there any justification with this testing?
Would auditors expect Pseudomonas testing?

Micro lab Air Pressure Monitoring

We currently monitor pressure differentials in the micro lab where we test non-sterile material. The test suites are under positive pressure. We work with agents that are BSL 2 or lower in a biosafety cabinet.

Is it a regulatory requirement to maintain pressure differentials in micro labs that are lower than BSL 3? I did not find any reference documents that suggest this.

A1: Maintaining the differential pressure in the clean room is a basic requirement . Room with higher air cleanliness should have positive pressure differential of at least 10-15 pascal with adjacent room of lower/ differing classes.

A2: Where do you get such an idea?

I checked the CDC Biosafety in Microbiological and Biomedical Laboratories Handbook 6th edition for Biosafety Level 2 and it states "There are no specific requirements for ventilation systems. However, the planning of new facilities considers mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside the laboratory.”

A3: My interpretation to [name redacted] questions was that she is asking about room DP not for BSC and the clean room in which BSC is kept could be used for other activities also.

I do agree [name redacted] that there is no requirement to monitor DP if BSC is used only for testing purposes and that particular room is not used for other microbiological activities where there is a requirement of clean room

Disinfectant Efficacy Testing

Has anyone performed Disinfectant Efficacy Testing according to AOAC Official Method 960.09 Germicidal and Detergent Sanitizing Action of Disinfectants?

We are currently trying to perform this and had some issues with some strains:

- While using *Staphylococcus aureus* as our challenge strain, the plate count was way higher than anticipated (using a 2McF sol.).
- Same happened using *Salmonella typhimurium*.

At first we thought our plates got contaminated since colonies were characteristic for both strains cited but with some smaller large colonies within. We repeated several times and got to eliminate contamination from: Petri dishes, neutralizer, agar, buffer, disposable material, etc.

Any thoughts? I've attached the Method and pictures.

A1: I just have a minor recommendation:

1. When in doubt get a definitive count. By that I mean the process of doing a serial dilution set to verify your inoculum levels to ensure you are not over spiking your test and that the rapid method for inoculum spiking in the AOAC method you are citing correlates to you analyst's methods and ability to reproduce.

A2: The AOAC Official Method 960.09 is not appropriate for disinfectant efficacy evaluations as it is a suspension test for food-contact surface sanitization chemistries. Staphylococcus aureus, ATCC 6538, does have characteristic white and gold colonies, but the images appear to have possible outside contamination present in both cultures, which could be driving the higher than expected counts. Further investigation into the potential contamination would be valuable using a longer incubation time to get better colony formation and then subculturing and using staining of the different morphologies to determine if the contamination is still present.

A3: My experience has been very satisfactory in the efficacy of disinfectants following the guidelines of:
Suspension methods:
EN 1040:2005 / EN 1276:2009/ EN 1650:2008 AND EN 13704: 2002
Surface methods:
EN 13697:2015
EN 16615:2015
AOAC methods are not suitable for use in the pharmaceutical industry.

Sterility of radioactive parenterals

I have a general question and seeking insights and opinions. I know many of you have experience in this field.. Even if not, for any pharma microbiologist out there In your opinion, why do you think the agency would be inclined to go absolutely ninja on the sterility assurance issues, contamination control issues, etc, for a product that is highly radioactive by nature, and has a shelf life of less than a week? While there is

no hard / objective data available to support the following statement, it is unlikely that infectious bacteria originating from a highly closed / isolated process would survive in the radioactive solution.

Is there a specific philosophy employed by FDA that guides these positions? They don't seem to be consistent. I was reading the other post re: testing for BCC over 0.6 Aw, and led me to question why FDA would go what really seems to be over the top (as was my observation) in assuring the sterility of a beta-emitting parenteral that would be expected to be highly anti microbial in nature to begin with? I'm guessing more anti microbial than a product with a 0.5 Aw. Don made a point that low water activity products still provide an environment where microbes can survive (even if they don't typically proliferate at an Aw of less than 0.6), which leaves some residual concern. With a radioactive solution though, I don't think this can even be said. I'm not sure what a residual concern would even be here, but this is the first radioactive parenteral I've worked with in over 20 years in industry (mostly aseptic parenterals).

A1: Deinococcus is found in the environment and I know customers who've isolated it. It's considered one of the toughest bugs out there. Other Bacillus an resist radiation as well. I worked with JPL to create a detection system for Bacillus in under 8 hours because that was the organism they were most worried about when sending our spacecraft into space. They were worried about us infecting space! I don't have experience with how much radiation is lethal but I've attached a study that talks about it.

https://news.cornell.edu/stories/2009/10/research-reveals-key-worlds-toughest-organism

https://pubmed.ncbi.nlm.nih.gov/16907802/

A2: Keep in mind that the sterility test is just an additional component of the overall monitoring/testing program to demonstrate the state of aseptic processing and effectiveness of contamination control strategies employed. You don't always perform the test for just a single batch, we test and monitor for the overall state of control as well.

I would point out that sterility is a requirement as per 21CFR361.1 and 21CFR212.70. You can also review FDA guidance document on GMPs for PETs.

Unfortunately, we cannot simply state that "just because it's radioactive drug means contamination isn't an issue", which shows me that you've never seen the toxic avenger movie (not to mention, the spider that bit Peter Parker was radioactive!). You're stating that it is "unlikely" that infectious bacteria would survive, yet there is evidence that some microorganisms do (Deinococcus, which albeit, I clearly don't expect to recover in a cleanroom).

With a high enough titer of contamination, or adventitious agents that are not specifically evaluated for, you could have a major problem despite being a radiopharmaceutical. These are still parenteral administrations and should be treated as such.

A3: It is a widely accepted fact within the radiopharmaceutical manufacturing community that radioactive drugs (radiopharmaceuticals) are not self-sterilizing.

Although high radiation fields are present and many radiopharmaceuticals do exhibit short half-lives that limit their effective use these parameters can not exclude the potential presence of bacterial contamination. The effective radiation fields in of themself are not sufficient for validatable sterility assurance of a complete drug container closure system.

Grade A air supply interpretation and requirements

In the draft for Annex 1 ithe following definition is given: "Grade A air supply – Air which is passed through a filter qualified as capable of producing Grade A non-viable quality air, but where there is no requirement to perform continuous non-viable monitoring or meet Grade A viable monitoring limits"

Would very much like to hear how this is interpreted in relation to setting the required action levels for total airborne particle level for ‘in operation’?

Should it be grade A levels to continuously verify the right quality of the produced air or follow the assigned grade of the area?

If you e.g. has a grade B cabinet or isolator with grade A air supply it is clear that the microbial levels ‘in operation’ should follow grade B requirements - but should the total particle level for routine monitoring follow grade A or the assigned grade B?

Does it have an impact on whether it is a cabinet or isolator?

And should same levels be used for total particle routine monitoring as for classification testing?

A1: The establishment of non-viable particle monitoring is established by the company based on the quality and integrity of its filters.

In most places where I have worked, aseptic areas have particle counters attached at critical points with continuous measurement.

In the case of isolators or biosafety cabinets, the classification specifies what to expect during particle monitoring, we cannot expect better results when it specifies ranges.

Purified water system performance qualification

In Purified water system performance qualification, we are following the strategy through testing of samples in 3 phases. Samples were collected from all the user points as two sets for 60 days covering one set alternative day, as a part of Phase-1 and Phase-2, generating data for 15 days from each sample point for each phase. The system was monitored for 1 year, as a part of Phase-3, with a frequency of monthly for the user points.

The regulatory expectation is to sample user points daily for 2 to 4 weeks (consecutive day sampling), as a part of Phase-1 and Phase-2, and weekly on rotation for 1 year, as a part of Phase-3. This requirement is conspicuously mentioned for WFI in regulatory guidelines and similar approach is expected for PW.

Concern: In our water system total sampling points are 75 No's for PQ and other than these regular schedule samples are about 35 No's. In this case sampling and testing of all 75 samples daily for 2 to 4 weeks (consecutive day sampling), as a part of Phase-1 and Phase-2 along with 35 regular samples is practically difficult.

Requesting to suggest the practical approach for water system PQ.

Regarding Bacterial Endotoxin Limit

Please suggest that all the Bacterial endotoxin limit of injection products in United State Pharmacopoeia is based on K/M formulae.

A1: I am hesitant to say "All" but if we are considering just small volume parenterals then the Endotoxin Limit (EL) would be calculated as K/M in most if not cases. The variables enter the equation in making sure you have the K and the M correct.

If you are uncertain about this you might want to consider this publication:

The Bacterial Endotoxin Test a Practical Guide edited by Karen Zink McCullough

This is easily obtained on Amazon.

There is a wealth of information in this book concerning the BET.

A2: I want to second [name redacted] recommendation This is a great book to have on hand. That said, I have been hearing that the FDA is pushing back on the limits and asking for even tighter limits on drug products, apparently based on the process capabilities.

A3: let me add to the previous replies that, according to USP 1085, you should always try to verify if the limits in USP monographs is "correct" (fit your dosage/route of administration, safety risk factor, etc) or not. See below what in USP 1085.

Some USP product monographs have endotoxin specifications defined at a targeted concentration for administered product. However, endotoxin limit specifications should be calculated for all indications in the product's package insert because indications and administrations for the product may be different from the data used to calculate the original USP monograph limit. If a form's most stringent limit is lower than the USP limit, the form should use its lower calculated endotoxin limit.

A4: Now my concern is i have a sterile lyophilized powder 100 mg in a vial and not a pharmacopeial article. Firstly reconstitute in 20 ml WFI so concentatuion will be 5 mg per ml. as per product literature for iv infusion administration further dilute in 0.9 percent sodium chloride so that final concentration will 1.25 mg per ml. if means we requier further 60 ml saline.

maximum dose is 5 mg per kg per hr.

So endotoxin limit will be 5Eu/kg/hr divided by dose so limit will be 1 EU per mg.

Now my concern is can we allocate sodium chlotride limit i.e 0.5 eu per ml. So we require 4 ml saline volume for 5 mg drug and 5 mg drug have endotoxin limit 5 EU.

4 ml saline have 2 eu

so 5-2 = 3 Eu per 5mg or 0.6 Eu per mg.

Now pharmacopoeia say that consider only active ingredients dose. than 1 eu per mg or 0.6 eu per mg which endotoxin limit will be correct.

Saline limit will be consider or not . if use limit will be stringent. please suggest

Storage period of Working microbial culture

We have procuring lyophilized vial of standard microbial culture from National culture collection center.we have Prepare working culture from it & maintain them at 2-8ºC for 6 months.We did not perform any validation for the storage period,it was decided on the basis of the cultural response we get during the six month.Is it necessary to perform validation ? or Is there any standard which describe the storage condition of working culture?

A1: Hi Shoaeb. Have a look at USP 1117, Microbiology Best Lab Practices for information on storing microbes. Also, here is a good article in general about long-term storage of microbes. Anecdotally, I trained people for years with the same microbes under -80C in glycerol and never saw any growth and/or ID problems.

https://microbeonline.com/preserve-bacterial-culture-long-time/

Microbial examination

The General Chapter " Microbial examination of non sterile products” *The* *Interpretation of the Results for Acceptance criteria for microbiological quality should be interpreted as follows:*
10^1 CFU: Maximum acceptable count 20
10^2 CFU: Maximum acceptable count 200
10^3 CFU: Maximum acceptable count 2000, and so forth…

*Now, please clarify t**he above* *Interpretation of the Results *is applicable for *Membrane Filtration, Plate count methods (Pour-plate method and Surface probable method), and Most probable number method or only for Most probable number method.*

<1229.5> Biological indicators for sterilization - update August 2022

the update to this chapter has the following

BI Manufacturer's Responsibility
The responsibility for determining the performance characteristics of each BI lot resides with the BI manufacturer. The manufacturer should provide, with each lot of BIs, a certificate of analysis that attests to the validity of BI performance claims. The manufacturer should provide information concerning the microbial population and resistance (D- and z-values, respectively, where appropriate) as well as storage and expiry information. The BI manufacturer ▲should▲ (USP 1-Aug-2022) include survival ▲and▲ (USP 1-Aug-2022) kill times for the BI in their documentation ▲and this should be verified by the end user. (USP 1-Aug-2022) T

Where it says 'and this should be verified by the end user' is this just a paper based review of the CofA and documenting this to state that it is included or must the end user perform sterilisation cycles to obtain this information? and if so how?

Unknown organism on MALDITOF

What must be done in order to add an unknown organism to the library and be able to use it like it is from the suppliers validated list?

If you are unable to obtain a result with the required confidence from the system by performing multiple attempts at the different techniques available (e.g. direct to extraction), however have sent this organism to another laboratory and they have performed analysis using a different technology (MicroSEQ) and obtain a result that either

1. Meets the system requirements and is listed within the validated list.
2. Did not fully meet the confidence level, however a review of the EMBL public database found a 100% similarity.

I have listed the two situations for opinions please as I assume that they could have different expectations.

Integrity Test

I have a problem with integrity test. I use Sterile Millipak®-200 Filter Unit 0.22 µm to filter paraffin.
I flushed the filter with water/IPA (70/30 ratio) and run the test. The pressure went up to approx. 2900mbar(it should be ≥3450 mbar) and then the test was stopped.
Any ideas why I cannot reach 3450 mbar.

Post sampling storage of EM plates

For companies that send their EM plates out for incubation, I believe the common practice is to refrigerate the plates after samples are taken until they are shipped. Shipment then commonly uses some sort of cooling pack.

I have read a study (specifically, https://www.usmslab.com/effect-of-usp-sample-storage-conditions-on-microbial -recovery-from-tryptic-soy-agar-tsa-plates/that) that states that this common practice can cause a lower recovery of both bacteria and fungi. Considering refrigeration does not stimulate growth but rather sustain it (within reason), this makes scientific sense. However, when I look at contract testing laboratories storage and shipment instructions, they consistently list the "common practice" described above.

What is the general consensus? Do you store at room temperature post sampling pending shipment and then do you ship cold? Has anyone done studies that confirm either as a suitable method?

A1: Generally, you can use a shipper that keeps the storage temp at 10-25 degrees and simply overnight to a lab (maximum 2 day shipping).

The study that Dina linked to, uses freshly inoculated plates which would be vastly different than in-situ EM samples (not to poo poo on that study FYI) that need to be incubated and enumerated. Cold temperature storage would be more deleterious to in-situ samples than fat, fresh, healthy inoculated (thing GP testing) plates.

I always recommend 10-25 degree shipper as it represents the lowest level of temperature shock to microorganisms. If you cannot feasibly get it to a lab under that temperature condition in a reasonable amount of time, find a closer lab or bring the micro testing incubation in-house.

Micro EM is a critically important assay and should always be treated as such.

A2: Now a day's portable incubator are also available for the storage of short period of time and they are used for transportation purpose only. You can use them.

In addition to that you should also have the transport validation of your existing practice for. eg. You can min c the monitoring procedure in the ideal conditions and you can validate your procedure by performing growth promotion testing.

Sterile medical devices

How long do you incubate medical devices for sterility test? 14 days or less?

A1: Hi. 14 days is the expectation unless you're able to validate an alternative rapid sterility method.

Is Bacillus cereus FDA's newest Objectionable Microorganism Target?

Received an interesting request from a client this morning. They forwarded a microbiological request from the FDA for a granular API that will be part of a solid oral dosage form (I'm assuming from a deficiency letter as part of an ANDA. The request stated:

> "Add additional in-house test, Bacillus cereus in Microbial Limit Test"

I do understand that the agency has had a few issues with B. cereus contaminations recently (Recall of RevitaDerm Wound Care Gel Due to Bacterial Contamination https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/blaine-labs-issues-voluntary-nationwide-recall-revitaderm-wound-care-gel-due-bacterial-contamination) but to request this for solid oral dosage forms seems overkill. I've discussed with

the client to let them know that there are no USP tests specific to B. cereus, however, I seriously doubt that this organization will push back.

I know that the problematic issues with B. cereus in food occur typically at higher concentrations:
From Australian food standards: https://www.foodstandards.gov.au › publications › Documents › Bacillus cereus.pdf

“Epidemiological evidence suggests that the majority of outbreaks worldwide due to B. cereus have been associated with concentrations in excess of 105 cfu/g in implicated foods. Rare cases of both emetic and diarrhoeal illness have been reported involving 103 –105 cfu/g of B. cereus in food. These cases occurred in infants or aged and infirm individuals (Kramer and Gilbert 1989; Becker et al. 1994). Laboratory studies on the formation of emetic toxin in boiled rice cultures support this finding, with >106 cfu/g of B. cereus required for toxin production to occur (Finlay et al. 2002). The use of a threshold is analogous to the No Observed Adverse Effects Level (NOAEL), commonly used in the assessment of risk from chemical substances in food. The threshold of 105 cfu/g is at any point after cooking, and not just the final concentration as used by McElroy et al. (1999) (described below)."

And FDA: https://www.fda.gov/food/laboratory-methods-food/bam-chapter-14-bacillus-cereus

“Bacillus cereus is an aerobic spore-forming bacterium that is commonly found in soil, on vegetables, and in many raw and processed foods. B. cereus food poisoning may occur when foods are prepared and held without adequate refrigeration for several hours before serving, with B. cereus reaching >106 cells/g.”

Just seeing whether anyone has seen other organizations that have received the FDA B. cereus request and whether there are any additional resources for limits testing? We are planning on adding the microorganism as part of the TAMC USP <61>, Suitability of the Counting Method as of now but we would be open to other suggestions.

A1: I concur that the concentration of B. cereus must reach high levels to illicit a clinical response, but it is the diarrhea and emetic toxins that are of most concern. However, depending on the process conditions, including heat and extremes in pH, the toxins could be adequately inactivated. But you still need high levels of the vegetative cells to generate the toxins. As you stated, for foods, toxins can be produced when the organism proliferates in food over time, or after being ingested, but again, at very high levels that I doubt would ever occur in your situation.

Unless there is a history of B. cereus in the product or process, and that presence has caused issues, I don’t see why you could not perform an assessment that concludes a low risk to the product and patients/ consumers taking the product. I have performed a number of these with little push back.

But without fully understanding the reason for FDA’s request for an API to include testing for this organism, it is difficult to conclude one way or another if this is a one-off situation versus a shift in regulatory expectations.

A2: I would like to add my 2 cents to your discussion.

Since 2011, one of the organisms that had been causing imported United States product detentions is the presence of Bacillus cereus in cosmetic products (See Import Alert #53-17 for Detention Without Physical Examination (DWPE) for microbiological contamination of cosmetic products). Generally, the infectious dose of Bacillus cereus is greater than 10e3 CFU/gram according to the scientific literature. However, most people feels that a low-level contamination of Bacillus cereus in a finished product (e.g. <100 CFU/ gram) does not constitute a risk to the health of a consumer because it is ubiquitous in nature and can be found in many types of soils, sediments, dust, plants as well as insects and a wide variety of foods. Besides, Bacillus cereus has been isolated from the conjunctiva of healthy individuals without any issues. Furthermore, I suspect that it will be highly unlikely that high levels of Bacillus cereus (e.g. > 10e2 CFU/ gram) will be detected in a nonsterile test sample.

There are many issues in identifying a recovered isolate as Bacillus cereus. In Chapter 14-Bacillus cereus of the FDA Bacteriological Analytical Manual (BAM), an identification schematic is provided to determine whether an isolate obtained from foods is Bacillus cereus . It is recommended in this chapter that the following tests such as motility, rhizoid growth, hemolytic activity and a test for protein toxin crystals be performed to differentiate members of the Bacillus cereus group into individual species. Based upon the literature, these tests are not confirmatory to determine a specific identification of a member of the Bacillus cereus group, but a presumptive identification. For example, most strains of Bacillus cereus and Bacillus thuringiensis are motile and hemolytic in Table 1 of this chapter. Most Bacillus cereus strains are beta-hemolytic. However, there are a few Bacillus cereus strains that are also nonmotile and non-hemolytic. It should be noted that Bacillus thuringiensis and Bacillus mycoides are also beta-hemolytic. It is also indicated in Table 1 of Chapter 14 that Bacillus anthracis is also nonmotile and nonhemolytic, but it is known that Bacillus anthracis strains AS182 and ASC 185 are both hemolytic. As for rhizoid growth of microbial colonies, Bacillus cereus strains will produce microbial colonies that will have a rough-galaxy shape which can easily be mistaken for rhizoid growth that is common for Bacillus mycoides. However, non-rhizoid variants of Bacillus mycoides have been isolated from the environment. The tests recommended in Chapter 14 of the BAM may be inadequate for distinguishing an atypical strain from culturally similar organisms such as Bacillus thuringiensis, Bacillus anthracis, Bacillus cereus and Bacillus mycoides.

Bacillus cereus is part of the Bacillus cereus group that consists of Bacillus anthracis, Bacillus cereus, Bacillus mycoides, and Bacillus thuringiensis. It is speculated that each of these species is a single species and each of these microorganisms are either a pathovars of a single species or a subspecies. From a genotypic and phenotypic perspective, it is not possible at this time to have 100% confidence in an identification call of a recovered Gram-positive bacilli isolate as Bacillus cereus. Biochemical, selective/ differential agars, fatty acid analysis and 16S rRNA sequencing identification techniques have problems in identifying a recovered isolate just as Bacillus cereus.

To detect a count of Bacillus cereus group besides a general bacterial count in a test sample, I suggest that you would additionally use either Bacillus cereus Selective Agar or Brilliance Bacillus Cereus Agar in addition to TSA of USP Chapter 61 since there is no USP Chapter for enumerating the number of Bacillus cereus group in a test sample and it will be difficult to differentiate this group of bacteria from others just by using TSA even thorough that they will grow on TSA. However, it should be noted that both Bacillus cereus and Bacillus thuringiensis will appear identical on each of these selective/differential agars, but at least you somewhat presumptively identify them during enumeration. I know that there will be individuals who will object in using a selective/differential agar as a microbial count agar, but I'm only suggesting the use of either Bacillus cereus Selective Agar or Brilliance Bacillus cereus Agar as an aid in determining a count of this group in a test sample. Based upon experimental work that I had conducted in the past, it would not be unexpected that low levels of Bacillus cereus will be detected in a nonsterile test sample by using either of these selective/differential agars.

A3: Just a follow up to Don's comment about "Bacillus cereus is part of the Bacillus cereus group that consists of Bacillus anthracis, Bacillus cereus, Bacillus mycoides, and Bacillus thuringiensis. It is speculated that each of these species is a single species and each of these microorganisms are either a pathovars of a single species or a subspecies. From a genotypic and phenotypic perspective, it is not possible at this time to have 100% confidence in an identification call of a recovered Gram-positive bacilli isolate as Bacillus cereus. Biochemical, selective/differential agars, fatty acid analysis and 16S rRNA sequencing identification techniques have problems in identifying a recovered isolate just as Bacillus cereus."

It is possible to sequence a housekeeping gene in order to differentiate between members of the Bacillus cereus group. Some contract labs offer this as a service if you need confirmatory testing.

A4: Hi, I saw recently where B cereus can express B. anthracis virulence genes and even cause some manifestations of Anthrax disease - this might have something to do with the increased concern. See below:

https://www.upi.com/Science_News/2021/10/18/Gulf-Coast-welders-dying-from-anthrax-like-disease-researchers-say/8331634571999/

“According to the report, 2020 saw two cases of anthrax pneumonia in welders caused by a rare B. cereus bacteria that contained anthrax toxin genes usually associated with the bacteria that causes anthrax. One patient died.”

A5: I’m just curious about which housekeeping gene that you are sequencing to separate each of the members of the Bacillus cereus group. Based upon the article by Caamano-Anelo, S. et al. in Food Microbiology 46 (2015), 288-298, it is my understanding that the housekeeping gene of tuf is a good target for differentiating B. subtilis and B. cereus from other closely related species. However, it is also stated in this article that the discrimination between B. cereus and B. thuringiensis is quite difficult despite the variability within the housekeeping genes of tuf, rpoB and gyrB genes based upon the figures that are present in this particular article.

A6: I can recommend this poster that was shared at PDA Micro in 2019: https://www.criver.com/sites/default/files/resource-files/SP-PDA-19-Species-Differentiation-Bacillus-Cereus-Group-using-the-pycA-Gene-Sequence.pdf

Our scientists found that pycA was the best gene target for differentiation. The poster has resources and publications listed if you want to read more.

A7: Just wondering ... now that whole genome comparisons using ANI, AAI and digital DDH are used in prokaryotic taxonomy has there been any published study(ies) that compared multiple strains of the various species of the Bacillus cereus group using these analysis methods?

CEHT

For CEHT (clean equipment hold time) study Non-sterile facility, the equipment is used in ISO-8 area can we follow ISO-8 limits i.e 50 cfu/25 cm2 or is there any specific limits for this.

If the equipment used intermediate stage in unclassified area, CEHT study required, if required which limit can be followed.is there any specific guideline or any reference documents available, any one following different approaches please provide.

Question about USP <1111> acceptance criteria

Can someone please tell me upon what rationale the following statement (found in Chapter 1111 and elsewhere) is based? I find nothing in FAQ or elsewhere as to how 10^1 = 20 versus 10, etc.

A1: Hi Deborah. I believe this is along the lines as a factor of two for growth promotion due to variability in the micro methods. At 10^1, that would mean you should expect anywhere between 1-20 CFU based on microbial test variability.

A2: As mentioned USP general chapter <1111> 10^1 CFU Means If the microbial level is 10 the maximum acceptable limit is 20 as per factor of 2 calculation.

Octenidine

Have anyone validated the micro method for analysis of octenisept or any product containing octenidine? What neutralizers did you use?

A1: Here you go! https://academic.oup.com/jac/article/65/8/1712/743725?login=true

TYMC and Bacteria

I'm assisting a colleague with a problem in regards to USP <61> TYMC when bacteria grow on SDA. Both my colleague and I are well aware of the USP text which says to count bacteria as part of TYMC and that you can add antibiotics to SDA if you expect to exceed TYMC specification due to bacteria.

With that said, here is my colleague's situation: for a particular material TAMC limit is 1000 cfu/g and TYMC limit is 100 cfu/g. The material has a good history of meeting these criteria. However, a recent lot was tested and bacteria colonies recovered in the TYMC test exceed the TYMC specification, 200 cfu/g. There was no growth on the TAMC plates. The recovered TYMC bacteria colonies were identified as Weizmannia (Bacillus) ginsenghumi. Attempts to subculture the TYMC colonies onto TSA were unsuccessful, even with an extended incubation. It apparently will grow on SDA but not TSA, which would explain why it was not observed in the TAMC test.

We are looking for the best path forward with material disposition- can this material ever be released or is it headed for the bin. As it stands, the material is non-conforming for TYMC, but only due to bacteria at a level that would pass TAMC. If you have experienced a similar situation, how was it handled?

Testing this material using SDA with antibiotics is problematic at this point since my colleague's test method has been filed with a regulatory agency and cannot be changed on a whim. In hindsight, it seems SDA with antibiotics should be used for all TYMC testing to avoid this situation.

A1: B. cereus and other Bacillus spp are susceptible to chloramphenicol hence it is common choice for inclusion in SDA to suppress the growth of spore-forming bacteria. The growth of Bacillus spp on Mannitol Salt Agar may be a problem too.

A2: What type of sample exceeded counts?
What is the nature of your product?

The fact that you had that level bacterial And yeast counts in general suggests an unsanitary condition. Possibly a very unsanitary condition.

A3: It is very possible that the strain of Weizmannia ginseghumi from SDA did not grow on TSA was due to the difference in the pH ranges of TSA and SDA for growing microorganisms. I found that sometimes bacteria that were able to grow on SDA could not be subcultured onto TSA.

A4: I've read a few answers to the question below, but it seems I may have missed how the disposition of the material should be addressed. By the time you recovering bacteria on SDA during TYMC, isn't it a bit too late to add the antibiotic being recommended?

Could it be justifiable to reanalyze the material with SDA that includes the chloramphenicol? If it was acceptable to use the modified TYMC media in the first place, I struggle with how a second analysis wouldn't be acceptable.

A5: Here's my interpretation of the data. The specs are as follows: "TAMC limit is 1000 cfu/g and TYMC limit is 100 cfu/g. The material has a good history of meeting these criteria. However, a recent lot was tested and bacteria colonies recovered in the TYMC test exceed the TYMC specification, 200 cfu/g". Yes the material exceeds the TYMC spec but the organism is a bacterium not a fungus. Therefore the TAMC spec applies (1000 CFU/gm) and the material passes this spec. Why it grows on TYMC and not TAMC is another question for another day and the answer should not affect product disposition.

A6: Likely pH the controlling factor. Soybean Casein at pH ~7 and SDA at ~5. Similar observations with a mildly acidic (pH 5-6) household product contaminated with Gluconoacetobacter - no growth on TSA at pH ~7 and TNTC on SDA.

Purified water pathogen testing

1. Do we need to test for specified organisms per 100ml in purified water, potable water and Raw water?
2. Can we place the decontamination autoclave in the washing area?

City Water / Purified Water Microbial Testing

We use purified water to make non-sterile products. The city already tests for coliforms. I don't see any value in testing incoming water that is further purified before it is used.

What is the regulatory expectation for testing incoming city water for Total Plate Count and Coliforms?

A1: The coliform testing by the city/state isn't the same as the testing in your facility. I would consult USP 1231 for some guidance.

A2: Microbial test specifications from the United States E.P.A. for drinking water can be found at the following site: www.epa.gov/sites/default/files/2016-06/documents/npwdr_complete_table.pdf.

In general, most people will conduct periodic microbial testing (e.g., heterotrophic plate count, total coliforms and fecal coliforms/E. coli) of the incoming drinking water from a municipal source on a periodic basis for only informational purposes unless they are obtaining potable drinking water from a well for their purified water system on the manufacturing site. If obtaining incoming potable water for the purified water system is from a manufacturing site well, microbial testing should be conducted on a routine basis to ensure that the source water complies with the microbial requirements for potable water.

E.P.A does not have a heterotrophic plate count limit for drinking water according to the table above, but most people will use a limit of <500 CFU/ml. My question is what do you do if the microbial test results of the incoming drinking water has a heterotrophic plate count of greater than 500 CFU/ml and is positive for the presence of coliform bacteria. In addition, you must remember that the E.P.A. has a microbial limit for total coliforms in which no more than 5% of the 100-ml samples taken can be positive in a month for large water systems or one positive for small water systems in which less than 40 samples are taken per month and a zero acceptance limit for the presence of E. coli/fecal coliforms. Furthermore, microbial testing of drinking or potable water is performed at the water treatment plant site where it is generated to ensure that it is meeting potable drinking water standards and not along sites of the potable or drinking water distribution system. For this reason, I would recommend that periodic testing of the incoming water from a municipal water system be conducted for only informational purposes to confirm that the incoming water is in compliance for the absence of E. coli and fecal coliform bacteria if asked by a regulatory authority since the incoming water for a manufacturing site purified water system must be in compliance with potable water standards.

I have a funny story about the time that a client reported a positive E. coli test result for their incoming potable water to the municipal potable supplier and demanded that they disinfect the town's water distribution system.

A3: I'd like to echo what [name redascted] said, USP <1231> IS THE source you need to be looking at. It's a long chapter but has all of the different grades of water, qualifications, testing requirements etc.

You can buy the compounding compendium from USP which contains 1231 for $150.

A4: You can test for Coliform and E coli bacteria along with full verification on periodic basis like annually . A simplified microbial testing for e.g. TAMC on TSA/R2A could be done more often ,(quarterly) to detect early trend in the microbial quality of the water supply

which route of administration recommended for clostridium should be absent

Which route of administration recommended for clostridium should be absent, and share reference guidelines.

A1: One category, would be oral for infants Clostridia can cause necrotising enterocolitis (NEC) in infants.

A2: Too broad a recommendation. The USP/NF monograph for honey has an absence of Clostridium spp requirement. Oral liquid dosage forms that contain honey usually have a warning not to administer to infants due to infant botulism.

FDA Quality Matrix

The FDA is reviving this Guidance For Industry.

How many OOS results are invalidated in the QC Microbiology lab? <1%, 1%, 5%, or 10%?

Invalidated Out-of-Specification (OOS) Rate (IOOSR) as an indicator of the operation of a laboratory. IOOSR = the number of OOS test results for lot release27 and long-term stability testing invalidated by the covered establishment due to an aberration of the measurement process divided by the total number of lot release and long-term stability OOS test results in the current reporting timeframe.

Escherichia coli Product Recall

I’m in agreement with previous statements by [name redacted] of [name redacted] that you can learn a lot by reviewing FDA product recalls. In reviewing 2021 cosmetic product recalls, I came across a cosmetic product recall of a Baby Lotion that had been found to be contaminated with Escherichia coli. I have found this to be distributing because it is indicative of a hygienic failure somewhere in the manufacturing facility.

In looking at the recalled product label, the ingredient listing are as follows: WATER, ISOPROPYL PALMITATE, GLYCERIN, GLYCERYL STEARATE, CETYL ALCOHOL, STEARIC ACID, CETEARYL ALCOHOL, DIMETHICONE, MINERAL OIL, OLEIC ACID, POLYSORBATE 20, MAGNESIUM ALUMINUM SILICATE, PHENOXYETHANOL, FRAGRANCE, CARBOMER, CAPRYLYL GLYCOL, XANTHAN GUM, SODIUM HYDROXIDE, PENTAERYTHRITYL TETRA-DI-T-BUTYL HYDROXYHYDROCINNAMATE, RED 33.

With exception of water, it is highly unlikely that the source of this Escherichia coli contamination in this finished product was from one of the chemical ingredients such as ISOPROPYL PALMITATE, GLYCERIN, GLYCERYL STEARATE, CETYL ALCOHOL, STEARIC ACID, CETEARYL ALCOHOL, DIMETHICONE, MINERAL OIL, OLEIC ACID, POLYSORBATE 20, MAGNESIUM ALUMINUM SILICATE, CARBOMER, XANTHAN GUM, SODIUM HYDROXIDE, PENTAERYTHRITYL TETRA-DI-T-BUTYL HYDROXYHYDROCINNAMATE, RED 33. Generally, these chemical ingredients are not susceptible to microbial contamination from organisms such as Escherichia coli. Phenoxyethanol and Caprylyl glycol are being used as the preservative system in this product formulation. Fragrances are antimicrobial due to the presence of hostile sub-ingredients in them.

Because low levels of Escherichia coli can be present in drinking or potable water, I wonder if potable water was the Escherichia coli contamination source that had been used somewhere during the manufacturing of this product formulation. I suspect that there are 3 possible root causes for this Escherichia coli contamination. One possible root cause is potable water that had been used during the cleaning of manufacturing equipment had not been removed from the equipment. A second possible root cause is that potable water had been used instead of purified water to remove chemical sanitizer residues after equipment sanitization. A third possible root cause could be that potable water had been used in the

manufacturing of this finished product. Because it is highly unusual to detect the presence of Escherichia coli in an aqueous cosmetic and drug product formulations, does anyone have a different idea of what could be the possible source of this contamination in this recalled product formulation?

A1: I am relatively new to this forum (after a long hiatus from pharma) so I thought I might chime in here.

Regarding the suggestion that potable water is the culprit. From your experience, are potable water sources readily available for use within the MFG vicinity? That has not been my experience.

I will suggest one alternative for consideration. Referring to the raw materials that you have outlined below, there are some, like Xanthan Gum, that have a specification for "ABSENCE" of E. coli and Salmonella in the USP (with good reason). There are probably a few more there. Xantham gum, in particular, is a bacterial fermentation product derived from plant/carbohydrate sources like corn. Natural grains like these are susceptible to pests at many stages of production. From the USDA inspection manual, there is an allowance for a certain level of adulterants (see snippet below from USDA grain inspection handbook with link) where 0.20% "animal filth" is the threshold; animal filth includes rodent pellets, bird droppings and insect excreta.

A2: In conducting cGMP audits of numerous non-sterile manufacturing plants in the United States and around the world, I have found that it is common to find a source of potable or drinking water in the manufacturing area. This water is often used for the cleaning of manufacturing equipment surfaces because the usage of deionized water is too expensive for rinsing aqueous product residues from manufacturing equipment surfaces.

For cosmetic products, there is no legal requirement that purified water be used in the manufacturing of cosmetic products that are not OTC drug monograph product formulations. Until your response, I was thinking that there was either an error in cleaning/sanitization of the manufacturing equipment or potable water had been used in manufacturing the recalled product.

Xanthan gum is often used in cosmetic products as a thickener. It is my understanding that Xanthan gum is often produced by using Xanthomonas campestris fermentation of a substrate such as hydrolyzed rice, barley and corn flour, acid whey, sugarcane molasses, etc., but glucose is used often used as a substrate because it is the best in terms of yield and product quality. However, there are companies that will instead use cornstarch or bean powder as the fermentation substrate. You may be correct that the possible source of the Escherichia coli contamination in the product formulation could be the Xanthan gum if cornstarch had been used as the fermentation substrate. Depending upon whether the Xanthomonas campestris fermentation broth had not been pasteurized by using 100-110C heat for 10-minutes to kill organisms before alcohol extraction with either ethanol or isopropyl alcohol, it is possible for Escherichia coli could survive in the gum. However, I would suspect that the levels of Xanthomonas campestris in the fermentation broth would suppress the growth of Escherichia coli in the fermentation stage, but it may be possible that some Escherichia coli could survive in the fermentation broth if pasteurization had not been used conducted. Generally, Xanthan gum will have a microbial count of less than 500 CFU/gram for the aerobic plate count. From testing numerous batches of Xanthan gum, I have come across microbial contaminants such as non-fermentative Gram-negative bacilli and mold, but I have not detected the presence of Escherichia coli in this material. The reason for not detecting it may be due to the difference in the fermentation substrate and whether pasteurization of the fermentation broth had been conducted before alcohol extraction. If the Xanthan gum is the source of the Escherichia coli contamination, it shows that a raw material from different suppliers may be different in their microbial quality based upon each of the suppliers manufacturing process. I have heard many stories in which the supplier for a raw ingredient had been changed to a different supplier because it was lower in price and now there are numerous microbial rejections from the new supplier and the reason for the out-of-specification contaminations was a different manufacturing process.

Personnel Monitoring Requirements

Our manufacturing process involves operators working inside of an ISO 5 BSC with the surrounding room being ISO 7. Operators are in sterile gowns with sleeve covers and gloves while working inside of the BSC. Operators are plated in the chest, forearms, and gloves after leaving the BSC. Employee who enter the room but do not enter the BSC are not required to be monitored. Is this methodology consistent with what is recommended in guidelines. I could not find a direct answer in my search.

A1: Without having seen your process, operator activities nor workflows, a simple yes or no answer cannot be offered. If you review the 2020 revision of Annex 1 draft guidance the expectation is that you sample operators upon each exit from the Grade B area.

I am more concerned with the fact that you take Grade A operator forearm and finger samples OUTSIDE of the BSC. How do you attribute any PM recoveries from the Grade A space and not as a result of sampling from within the Grade B space?

All BSC related PM samples should be taken inside the BSC before exiting Grade A and held to Grade A surface limits.

A2: I believe I disagree with [ma,e redacted].

Unlike air and surface samples, personnel sampling can be conducted outside the BSC or the cleanroom often when the operator is leaving the area. Two advantages supporting this practice are personnel sampling on exit would represent a worse case and you avoid additional interventions in the more critical area by the sampler.

A3: A firm is more than welcome to sample Grade A occupying operators anywhere, however the operators are still held to established limits for the BSC.

Sampling an operators fingers and sleeves outside the BSC doesn’t create a "worst case scenario", it creates a situation that shouldn’t exist. Operators should don sterile sleeves and outer gloves immediately prior to entry into the BSC and remain in there for the entire duration of the aseptic activities.

If you sample outside the BSC you may have a recovery that you would have to associate to a Grade A/ ISO 5 limits that did not actually occur from within that environment. There really wouldn’t be any additional risks associated with the sampling practice as the settle and surface plates would already be introduced into the BSC at that point in time when sampling occurs.

In contrast to your opinion, I would want to monitor Grade A surfaces in the Grade A areas in the instance there is a recovery. This would keep a majority of the resulting investigation related to the BSC activities (and perhaps material transfer).

A4: If I may add to this minor "squabble". I can see and agree with Marc's position of arms and gloves sampling within the Grade A if SOP allows for operator self-monitoring. For someone else to do the sampling, it becomes more nuanced and perhaps awkward for another person to reach operators gloves and forearms while they maintain them inside BSC.

I would consider if the process has both primary BSC "operator" and a second support "verifier" person in Grade B for PM sampling, then have the support person ready to take operator glove and forearm samples as soon as they exit the BSC before operator touches something or does anything else. The risk of Grade B area influence would seem extremely low given the short time of plate exposure.

A5: My comments did not address the limits but the sampling location. Naturally sampling representative of the controlled area would be evaluated against the limits for that area.

Sampling when exiting a controlled area gives an accumulative sample whereas sampling within a BSC would not define the timing of the operation.

A6: Regarding this subject...

How can we optimize air, surface and personnel monitoring in Blow-Fill-Seal systems?
Any recommendations regarding ISO 7 preparation area and ISO 5 filling area?

A7: I completely disagreed with [name redacted]

Simple is that i you sample in Grade A or Grade B, C so follow the Viable Surface limit of Grade A , B, C respectively.

A8: Not so simple. The work surface and the airflow remains in the BSC or Class A area while the operator moves. I believe that regulators like operators to be monitoring each time they exist in an aseptic processing area.

A9: Classification A and B have similar specifications in terms of 0.5 particles, varying in larger particles, although they have different classifications depending on the state of work, for this reason this discussion should be based on which state to perform the sampling.

In particular, we carry out a sampling before the beginning of the process and at the end of it, I would like it to be at the end only as Tony explains, since if you have validated and qualified techniques and methods, there is no greater risk that they escape from our hands. and what is inherent to the operator.

CHAPTER 81

I have a question related to antibiotics assay. USP says the diameters of the inhibition zones must be between 11- 19 mm and S3 standard must be from 14 to 16 mm , but I could not achieved this diameters. Have you had problems with this? By the other hand, with smaller inhibition zones it is not possible to meet the acceptance criteria of the product.

A1: If you are getting a smaller zone size which means the concentration of organisms in the agar is high and vice versa for bigger zones. I would recommend evaluating organism concentration between 2% to 25%at 580nm with the different amounts (For Example 1 to 10 mL) per 100 mL of agar medium to get correct zone sizes.

A2: Please share details of what is the sample and which positive culture was used for the study.

A3: It's been a while, but I tested antibiotics for a number of years. I didn't always get 14-16mm for the S3, but that is the "sweet spot." Zones too large (>20mm) will start to get too close to the plate wall for a good reading. And correct me if I'm wrong, but because this is a logarithmic equation, the small zones are too small for good precision and accuracy. In other words, small variances in the smaller zone sizes result in large changes to the end results.

As for what you can do about it, if the assay isn't yet validated, you can change some of the parameters to get the zones into the 14-16mm range, and then validate it with those parameters. Your best bet is to try making your inoculum slightly more dilute. And I think that's all you can do. I would not change the final standard/sample stock concentration to anything other than what the USP recommends. Also, I would not change the base layer agar or the seed layer amounts.

A4: From my understanding, it will be better to ascertain the following:

1. The potency of chemicals for the assay.
2. The concentration ratio for assay mixture.
3. The test status of the test strain (if it is a pure and viable strain).
4. The optimum physiological conditions for the assay.

USP 1117 _ under revision

As per USP1117 (which is under revision)

Section" sample handling " page No 6/ 11 describes" MONITORING OF WATER OR BIOBURDEN PRIOR TO BIOBURDEN REDUCING STEPS IR STERILIZING SAMPLES MAY BE HELD AR 2 TO 8 DEGREE CELSIUS FOR UPTO 24 HOURS FROM THE TIME OF SAMPLE COLLECTION UNTIL THE START OF THE ANALYSIS. WHEN THE TESTING WITHIN 24 HOURS IS NOT POSSIBLE (EG: CONTRACT LABORATORIES) THE ACTUAL MAXIMUM HOLD TIME BETWEEN COLLECTION AND TESTING SHOULD BE SUPPORTED BY EXPERIMENTAL STUDIES."

Query: Based on the above sentence , does it allows to establish hold time studies for buffers (microbiological parameters)which is used in biopharmaceutical manufacturing?

Monitoring of compressed air used in pharmaceutical manufacturing processes

is there anywhere that it states how you should select and position a sampling location? E.g to be as close to the actual point of use, after any final filtration.

I think that it is a basic expectation and inspectors look for this but i cant see anything to reference against.

A1: FDA's Guidance for Industry for Sterile Products Produced by Aseptic processing describes 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."

So although not specific where exactly to sample, there is the expectation that it is done after filtration. To reduce the risk of introducing contamination from a (lower) classified area it should be done in the area itself, or at least in an environment of equal air classification as the gas is introduced into.

Useful calculations in microbiology

Can anyone share some useful calculations that they use when calculating cfu/inoculum. (Plus anything else thought helpful)

For example;
If you obtain a number of cfu on a plate and need to calculate back to cfu/test solution. I do it a long way by calculating what my cfu were per ml, then multiply by dilution factor from my serial dilution and possibly initial product volume.

How can you easily calculate your dilution factor and inoculum volume if you state that you want to have a certain count e.g. 50cfu or 200cfu, from the original bulk solution with an approximate known cfu/ml. I have to work out an easy dilution series e.g. 1 in 10000 (by doing 1 in 10, four times), then factor in a 1 in 2 or a 1 in 5, and then my inoculum of 0.1ml would be correct.

I know there is an easier and standard way but it is a very long time since ive been doing it long hand. its simple micro related stats that i cant see anything when i try to look it up (i.e. Google or stats books)

Also, we don't use 100ml, 1000ml or any other specific volume diluent solutions and we have 9ml solutions to perform serial dilutions so we have been restricted. I'm looking to make it simpler for calculations and possibly reduce error.

A1: The C1V1 = C2V2 equation commonly used in chemistry can be applied to microbe suspensions. If you are unfamiliar: C1 is the starting concentration, V1 is unknown, C2 is the target concentration, and V2 is the final volume for the target suspension. Solve for V1 to determine what dilution is needed to get your desired suspension.

For example, if performing growth promotion you might want a suspension at 400 cfu/mL (100 µL delivers 40 cfu). That's your C2.
You might want a 10 mL volume of that desired suspension. That's your V2.
Through a standard plate count, turbidimetric measurement, or other means you determine the starting titer is 1.5×10^{9} cfu/mL. That's your C1.
$(1.5 \times 10^{9}$ cfu/mL) • V1 = (400 cfu/mL) • (10 mL)
$V1 = 2.7 \times 10^{-6}$ mL

If you perform six serial ten-fold dilutions (10^{-6}), then take 2.7 mL and dilute it up to the V2 volume (add to 7.3 mL diluent) you will end up with 10 mL of your desired suspension at 400 cfu/mL.

Of course, you could set up a spreadsheet that does all of the calculations for you to make it even easier.

B. cenocepacia

Is anyone else having difficulty with recovery during method suitability of B. cenocepacia? I have had no success recovering this organism in a nonsterile drug product (syrup) using tween, lecithin, >48 hour enrichment, and up to a 1:100 dilution. Of the three BCC organisms, I find this one to be the most problematic both for method suitability and growth promotion.

A1: Have you tried to increase the incubation time to 72 hours for the enrichment broth to detect the presence of Burkholderia cenocepacia? With some product formulations, I have found that the incubation time had to be increased from 48 to 72 hours to recover each of the Burkholderia cepacia complex test organisms that is indicated in USP Chapter 60..

In looking at the information that you had provided, it is unusual to use a 1:100 dilution of a product formulation. Do you need to use a 1:100 dilution of the product to recover Ps. aeruginosa, E. coli, S. aureus and Salmonella for the product formulation in your method suitability testing?

To understand as to why a 1:100 dilution of the test sample is being used, what is the pH and the preservative system in the product formulation that you are trying to conduct method suitability on? Furthermore, what is the concentration of tween and soy lecithin that you are using to perform method suitability in both the diluent and enrichment broth of USP Chapter 60? I have found that the usage of 4% Tween 20/80 and 0.5% Soy Lecithin in both a microbial count diluent and enrichment broth have been successful in conducting method suitability testing of test samples.

A2: With those neutralizers and a 1:100 dilution the issue is more likely to too short an incubation time.

A3: To echo [name redacted] reply, we have also noticed that sometimes these BCC strains require closer to the maximum incubation time. We have a couple products where we need to incubate both steps for the 72 hours.

A4: We performed a 1:20 and a 1:100 and yes, we did continue the enrichment incubation as well as the plate incubation for 72 hours, still with no recovery of B. cenocepacia from the product suspensions (but did have recovery from positive control - no product). S. aureus, E. coli, P. aeruginosa all performed fine at 1:20, as did B multivorans and B. cepacia. Salmonella was recovered at 1:10.

We repeated the validation for B. cenocepacia only, at 1:100, as that is the method we follow when we have difficulty with recovery, as we sometimes see with S. aureus.

pH is 4-5
preservative is 0.2% sodium benzoate

Phosphate buffer contains 10% Tween 20 and 0.5% lecithin.
TSB contains 4% Tween 20 and 0.5% lecithin

I am curious as to what is the maximum dilution other labs adhere to? We typically perform 1:20 simultaneously with 1:100.

Of note, this formula contains HONEY and as the product suspensions are turbid and likely overrun with Bacillus, I wonder if the bacillus is out-competing the B. cenocepacia. I streaked to TSA also and have a lot of growth but have not identified it yet - but not B. cenocepacia.

A5: I have found that shaking during incubation can help too.

A6: Try using Deys and Engley broth and agar for dilution and plating. Let me know the results once you try.

A7: If the other two Bcc species are recovered in 48 hours neutralization is probably not the issue so extending the incubation time to 72 hour should work and would be used routinely during product testing.

A8: In reviewing your response, I would say that you are having 2 issues. Without knowing the actual composition of the product formulation, I would say that you might still have preservative neutralization issues for demonstrating recovery of Burkholderia cenocepacia. It is very easy to neutralize the antimicrobial activity of 0.2% Sodium benzoate with the dilutions and Tween/Lecithin concentrations that you had indicated. The reason that I'm stating this is that you were able to validate either a 1:10 or a 1:20 dilution for the recovery of S. aureus, E. coli, Salmonella, Pseudomonas aeruginosa, B. multivorans and B. cepacia, but not for Burkholderia cenocepacia. It is my understanding that Burkholderia cenocepacia is a slow grower, but I'm not sure if this contributes to your issue in recovering Burkholderia cenocepacia from a test sample. By increasing the incubation time to 72-hours, it allows for the detection of Burkholderia cenocepacia that is not possible with shorter incubation times. Dilution of a test sample is one of the ways to neutralize the antimicrobial activity for some preservative systems. As far as a possible dilution, I would recommend that you increase it to 1:500 as long as a 1.0 gram test sample is being tested. If you still do not have neutralization of the preservative system at this 1:500 dilution, you may have to investigate the usage of other chemical preservative neutralizers for inclusion in the microbial count diluent and enrichment broth. Honey is a sugar and high concentrations of sugar can inhibit the growth of microorganisms by an osmotic effect. Without knowing the concentration of Honey in the formulation, I suspect that a 1:500 dilution may work for recovering the presence of Burkholderia cenocepacia.

The second issue that you are having is the presence of Bacillus species by using Honey in the formulation which may be preventing the isolation of Burkholderia cenocepacia due to it being a slow grower in comparison to Bacillus species. Honey has a USP APC limit of 1000 CFU/gram. To take care of the Bacillus contamination from the Honey that is used in the formulation, I would recommend that the order of addition of raw ingredients to the formulation be changed. I would recommend that a side kettle be used to add Honey to purified water and heat it to 60 to 80C for at least 30 to 60-minutes to take care of the Bacillus species that may be present in the Honey. After heating and cooling it down to room temperature, I would add this heated Honey phase to the remainder of the formulation. By heating to 60 to 80C, it should either self-sterilize or pasteurize the Honey in which there is no longer an issue of Bacillus being introduced into the formulation by using Honey as an ingredient in which Bacillus out competes Burkholderia cenocepacia. You may then be able to recover Burkholderia cenocepacia. This heating trick is commonly used by manufacturers to take care of biocontamination that is present in many natural raw ingredients that may have high microbial levels in which most people in Quality Control Microbiology Laboratories are unaware of using step in a manufacturing process.

Interesting FDA Observation

This observation in a Warning Letter is interesting, as it implies Burkholderia contamination would be objectionable in both water specifications and the water system. This company manufactures non-sterile products.

"No investigations were conducted in response to identification of Burkholderia contaminans in seven samples taken from your water system ports (b)(4), and (b)(4), between January 1, 2020, and June 30, 2020. Burkholderia contaminans was not identified as an objectionable organism in your procedures or your (b)(4) water specifications. The presence of objectionable microorganisms in your (b)(4) water system can adversely affect the quality of your drug products."

A1: This poses many questions:

Is the use of R2A agar for water monitoring and identifying the predominant isolates adequate for Bcc screening?

How aggressive must you be in identifying Gram-negative bacteria when conducting microbial enumerations of purified water, e.g. if the count exceeds the action level of 100 cfu/g, exceeds your internal alert level, or if you obtain any counts.

Gram-negative bacteria are the normal microflora of a water system. Does the FDA expect we exclude Gram-negative bacteria?

Identification of the members of the B. cepacia complex is not a trivial task and requires access to MLST.

Not all the 23 members of the Bcc are pathogens. Will some species be considered objectionable and others not.

Will the need to conduct an investigation be dependent on the non-sterile drug products you manufacture? No for compressed tablets, yes for oral liquids?

Purified water is not a drug product, so why would it have a specification?

Is the isolation of a Bcc member 7 times over 6 months an adverse trend?

If your manufacturing process mitigates risk, e.g., sterile filtration, heating the purified water or bulk solution to 60 degree C, can your purified water monitoring be less aggressive?

If your AET include B. cepacia as a challenge organism, can your purified water monitoring be less aggressive?

Plenty of questions but fewer answers!

A2: Any idea, if the company manufactures non sterile dosage forms or cleaning agents or creams. This is interesting to know as most of the non-sterile dosage forms are dry solid with minimum use of water and it will not support microbial growth due to their low water activity and unlikely to cause any spoilage...reference per USP <1112>.

A3: Unfortunately the answer is "yes" to most of them. That is the new expectation, based in compliance (not science).

A4: Hello, could you send the warning letter or the search code?

A5: While I cannot answer the regulatory questions on the FDA Observation, maybe some of the regulatory folks on the PMF list will provide some guidance.

When one of my clients had a similar observation, we ran studies and showed we would recover the BCC bugs equally on R2A media.

A6: This observation regarding the BCC is a part of Warning Letter # 614058. Just to add some detail to the discussion. The company cited manufactures OTC mouthwash products for the treatment of dry mouth among other oral care products. The warning letter explains that the products are treated as medical devices because "they are intended for use in the diagnosis of disease or other conditions or in the cure, mitigation, treatment, or prevention of disease, or to affect the structure or any function of the body."

Also, there are several other issues to be noted. The company opened a CAPA to address their BCC issue but closed the CAPA prior to actually implementing planned changes. There were excursions in the performance parameters of their water system that occurred while the system was being validated but they failed to mention those excursions in their water system validation report. The warning letter is quite lengthy with other observations.

If you go the company website and look up the product information for the mouthwash in question the ingredients are listed as "Aqua, Hydrogenated Starch Hydrolysate, Sodium Citrate, PVP, Propanediol, Betaine, Gluconolactone, PEG-40 Hydrogenated Castor Oil, Xylitol, Taurine, Sodium Benzoate, Citric Acid, Sodium Monofluorophosphate, Sodium Hydroxide, Sodium Hyaluronate, Sucralose, Aroma, Calcium Gluconate, Stevia Rebaudiana Extract."

This is clearly an aqueous product formulation.

Considering the BCC to be objectionable for an aqueous mouthwash doesn't seem unreasonable.

Gram negative rods in purified water

Are you allowed to obtain Gram negative rods within purified water used in pharmaceutical manufacture? I would assume that if a specific G-ve species is listed on the product specification then you would want to ensure absence of this and would not use PW to manufacture the product. or potentially asses if it could have been introduced into the product if the water monitoring location is not monitored at the time of batch manufacture.

Is this the case and what about other general G-ves that are isolated?

A1: Gram-negative, oxidase positive bacteria are the normal microflora of pharmaceutical-grade water systems. Most of them are capable of forming biofilms. The microbial count limits using plate count or R2A agar range from an indicative NMT 500 cfu/mL for potable water, to NMT 100 cfu/mL for purified water to NMT 10 cfu/100 mL for water for injection.

According to Tim Sandle the most common waterborne bacteria are *Burkholderia cepacia, **P. aeruginosa, **P. fluorescens,**P. alcaligenes* * P. **oryzihabitan, **Alcaligenes spp., **Stenotrophomonas spp., **Ralstonia picketti, **Acinotobacter lowffi, **Serratia spp.* *Flavobacterium spp. and **Brenvundimonas diminuta. *

The question that is discussed is whether as a risk mitigation you should exclude members of the B. cepacia complex from purified water use to manufacture aqueous, non-sterile drug products and how is this best achieved.

A2: In reference to your question, what type of purified water system are you talking about? For example, I have never isolated a Gram-negative bacterial species from a distribution loop that contains circulating hot water or water containing ozone. However, I have isolated Gram-negative bacteria from many ambient circulating distribution loops. In addition, it is also common to isolate Gram-negative bacteria from sand or multi-filters, water softeners, carbon filters, and ion exchange columns of the pre-treatment portion of a purified water system whether it has an ambient, hot or ozone distribution loop.

In regards to your question, I would believe that a regulatory authority would have objections to the presence of any Gram-negative bacteria that is obtained at a distribution use-point based upon the many FDA warning letters and 483's that have been issued to companies concerning their purified water systems. In general, the following Gram-negative bacilli species have been recovered from process water systems: Pseudomonas aeruginosa, Ralstonia pickettii, Pseudomonas fluorescens, Bradyrhizobium species, Sphingomonas species, Flavobacterium species, Burkholderia cepacia complex, Moraxella species, Stenotrophomonas maltophilia and Flavimonas oryzihabitans. The presence of any one of these Gram-negative bacterial species at a use-point in the distribution loop of a purified water system in my opinion would be objectionable. I will admit that I have never seen a fermentative Gram-negative bacterial species in a purified water system, but it could be a matter of luck in not detecting them. If you have an ambient circulating purified water system, I would be more concerned as to whether there are appropriate microbial control measures to prevent the introduction of Gram-negative bacteria from the pre-treatment portion of the purified water system in which the source is from the incoming potable source water for the system.

Finally, your frequency of sampling purified water use-points would have been determined during the validation of your purified water system to ensure that the best microbial quality is being obtained at all times in which the system is being used in manufacturing of finished product.

In conclusion, I know that there will be objections to my opinion that no Gram-negative bacteria should be present in the distribution loop of a purified water system, but I do not want to deal with product recalls due to Gram-negative bacterial contamination in finished product or a lengthy FDA inspection of the manufacturing facility in which each Gram-negative bacterial species that has been previously isolated from purified water samples is acceptable or not. No matter what, I will lose this argument concerning the acceptability of a Gram-negative bacterial isolate in a purified water sample to the FDA. Sometimes, fighting the FDA is like shoveling sand against the incoming tide and I would like to do more constructive tasks.

A3: There is the general problem with microbial test i.e. high lag time; from sampling of PW to testing and identifying organisms at least 5-7 days are lost. And the sampled water is already used in the production process.

I have once found gram -ve rods in PW used for tablet manufacturing. Conducted a profiling and risk assessment - was able to justify no impact on product by low water activity of product and non spore forming nature of isolated microorganism.

Still steps were taken to remove the gram-ve organism from the system by sanitization.

A4: [name redacted] it is a totally unrealistic expectation to have no Gram-negative oxidase-positive bacteria in a purified water distribution system.

You may recall the Seinfeld episode around the availability of the Today's Sponge. Elaine classified men as being sponge worthy. That product was deleted due to FDA observations about the water system in a South Jersey manufacturing plant. Art imitating life.

You can pretty much exclude them in an ozone-treated purified water system or a hot water WFI distribution system but not with periodic heat sanitization.

[name redacted], you are right that the discussion is mainly directed to aqueous, non-sterile drug dosage forms.

A5: I disagree with your opinion that I'm totally unrealistic in having the absence of Gram-negative bacteria in the distribution loop of an ambient temperature circulating purified water system. It all comes down to having a properly designed purified water system in which there is the presence of 2 or more different microbial control measures present to control the presence of Gram-negative bacteria in the distribution loop of an ambient temperature purified water circulating system. With the exception for the introduction of ozone or using hot water, these different microbial control measures should be used before and after the

purified water storage tank of the distribution loop. Generally, I have seen many ambient temperature purified water circulating systems in non-sterile manufacturing plants that have been improperly designed in the first place that allows the establishment of Gram-negative bacilli biofilms in which there is now the proliferation of Gram-negative bacteria to be present in the distribution loop.

Are you now indicating that the only acceptable circulating purified water systems are those in which circulated water in the distribution loop is either at a hot temperature or with ambient water containing the presence of ozone? FYI, I'm totally for the usage of these 2 systems in which purified water is circulated at either a hot temperature or there is the presence of ozone in ambient circulating water, but many companies have instead decided to go with ambient temperature purified water circulating systems because it costs less to design, build, maintain, operate and safety from due to exposure to either hot water or ozone. They will eventually pay for this mistake by not using a hot purified water circulating system or ozone is circulated in ambient purified water by having either rejected batches or a product recall of a non-sterile finished product due to the presence of Gram-negative bacteria if the system is not properly designed.

Furthermore, are you saying that having the presence of Burkholderia cepacia complex in the distribution loop of an ambient temperature purified water circulating system is O.K. for the manufacturing of non-sterile products because it is impossible not to have these systems to be free of this particular group of Gram-negative bacteria? If so, I think that you are being unrealistic in this aspect. I think that the FDA would agree with me due to the issuance of several recent FDA warning letters on this subject.

A6: A poorly designed water system/distribution loop can lead to contamination issues, regardless of whether the system is hot water or ozone sanitized.

The presence of dead legs and non-sanitary valves, use points or sampling ports re examples of design issues that can trap organisms, allow them to proliferate and neither ozone or hot water may completely eliminate or prevent biofilms. For example, you can send hot water through a system but if there are areas where the temperature cannot be maintained for an adequate amount of time, sanitization will not occur. I have seen this occur at sites with Gram-negatives, including BCC, in water systems and distribution loops.

A7: I'm with [name redacted] re. this matter. My experience is similar. Potential biofilm development by cepacia or Gram negative bacteria justifies affirmative measures such as ozone/UV and heat for relevant loops. Especially in context of high volume continuous production and intrinsically untimely culture monitoring, magnitude of at risk warrants investment in such affirmative measures .

A8: [name redacted], is this consistent with an industry limit of 100 CFU/mL. If a low microbial count is acceptable what would you expect the composition to be?

A9: Coming from an excellent conference in Charleston for Charles Rives last week AND hearing from multiple recent visits from the FDA with various clients in multiple states I can see both sides of this topic.

From discussions in Charleston and some of the questions that the FDA has requested of my clients I see several issues:

1. The FDA Investigators are VERY concerned about the microbiological quality of water regardless of whether the non-sterile manufacturer is an aqueous dosage form manufacturer, a mix of a solid and aqueous manufacturer, or solely a solid oral dose manufacturer. They expect ID's and appropriate testing for their water.
2. By appropriate testing, the USP <1231> recommendation of 1mL pour plate or 1mL membrane filtration onto SMA, 2 to 3-day incubation at 30-35C is not being accepted by Investigators WITHOUT applicable "suitability testing".
3. The term "suitability testing" I believe is being used inappropriately by the investigator. I believe they are looking for testing following USP <1231>, section 8.5 Test Methods suggestion noted below:
"Every water system has a unique microbiome. It is the user’s responsibility to perform method validation studies to demonstrate the suitability of the chosen test media and incubation conditions for bioburden

recovery. In general, users should select the method that recovers the highest planktonic microbial counts in the shortest time, thus allowing for timely investigations and remediation." Meaning a mix of test parameters to recover the highest cunts in the shortest amount of time (high nutrient vs low nutrient media; 0.2 vs 0.45micro membrane filters; 20-25C vs. 30-35C incubation and incubation durations such as 2, 3, 5, and 7 days incubation and enumeration).
4. Growth promotion testing of media used for water heterotrophic plate counts has been requested to show that the test methods are capable of recovering common water microorganisms.
5. Identification of recovered microorganisms specifically looking for Gram-negatives.

So my question is if you are trying to show your water system is absent of Gram-negative microorganisms and you routinely get <1 cfu/100mL (not unusual) does this mean you should test 200mL? 500mL? 1000mL? How far do you go to demonstrate that your system is "clean" if you have performed the "suitability testing" and growth promotion requested by FDA above? The investigators are apparently not accepting 1mL testing volume as your sole micro-test volume.

A10: I hate to say it but they are expecting the absence of gram negatives. As Don so eloquently stated, the only way to achieve this is with hot water (80C) or ozone or ultrafiltration. In essence cold PW systems are becoming obsolete.

A11: In reference to the 100 CFU/ml specification for purified water, it might be the industry standard because it is indicated in the USP Monograph for Purified Water. I believe that this purified water specification has been in place for 50 years. With various publications by ISPE, W.V. Collentro, T.C. Soli, and T, Meltzer concerning the design of purified water systems in the last 25-years, I wonder whether this purified water specification of 100 CFU/ml is even correct today based upon the actual test data that people are obtaining for their purified water samples from use-points of the distribution loop. It is my opinion that an industry standard for a purified water count should instead be based upon generated test data that is being obtained by many companies and not determined without supporting test data.

I have found that most of the time that purified water samples do not normally have this level of microbial contamination up to 100 CFU/ml. With a properly designed, operated and maintained purified water system, the microbial counts of water samples taken from the distribution loop has often been <1 CFU/ml. To increase the level of detection for the presence of microbial contamination, I had to increase the amount of purified water to be tested from 1 ml to 100 ml. With 100 ml purified water samples, the microbial count would still often be <1 CFU/100 ml at various tested use-points. If microbial contamination was detected by testing a 100-ml water sample from a use-point, the recovered microbial contamination level was often between 1 and 10 CFU/100 ml. Often, these recovered isolates were not Gram-negative bacilli, but were Gram-positive cocci and bacilli that properly came from during the sampling process by either the operator or exposure of the open sample container to air.

From reading previous posts, I have seen people recommend not to conduct identification of recovered water isolates unless the count was at or above the alert/action levels for the system. I do not agree with this practice in regards to the identification of recovered water isolates. If you are obtaining a count for a water sample, I would always conduct identification of the recovered isolate to the genus/species level no matter what is the count in order to detect the presence of an issue for a purified water system. Unless a biofilm was present within the distribution loop or there was an operational issue with the purified water system, I had never recovered a Gram-negative bacilli in routine distribution loop purified water samples because extensive IQ/OQ/PQ testing of the purified water system had been conducted to ensure proper microbial quality of water is being obtained.

I will admit that Gram-negative bacilli are normally present and at high numbers greater than 100 CFU/ml after multi-filters, water softeners, carbon filters, and ion-exchange columns of the treatment portion of the system. However, I'm not concerned about the treatment portion of the system because microbial control measures are normally present before R/O units and before the water storage tanks of the distribution loop.

A12: I am still enjoying this discussion by all involved and will need to assess all information and what final position and expectations we apply as there are some differences of opinion being provided.

However, if we take the understanding that within the allowable limit <100cfu/ml, we may obtain gram negative bacteria (even if this is 1cfu within 100ml or 200ml and think it feasible to be able to assess the species if obtained for their acceptance or not.

What would your opinion be for an aqueous nasal spray and how would you approach this?
We would categorically not allow Bcc or P.aeruginosa due to product type and specified organism expectations.

A13: With regards to the ambient systems using ozone do you have any pointers to guidance documents or operation please.
e.g. is ozone just used intermittently as part of an ongoing sanitisation program or is it permanently within the water but removed prior to usage as you need to remove any added substances.
any info on set up, design and maintenance would be greatly appreciated.

A14: I believe testing minimum 10mL (provided high bioburden based on historical trend) and 100mL for all water samples on a suitable medium will be a good approach. In addition, all growth (Even within specification) must be identified up to species level to ensure we document and approve objectionable organism evaluation form.

We must demonstrate by Method suitability that a suitable medium, time and temperature will allow slow growing organisms to recover.

A15: Are you aware of last century's Sinex contamination?https://www.sciencedirect.com/science/article/abs/pii/S0022354915443849the cepacia most likely was in the water system.

Question for the folks in discussion - are we not discussing the use of a raw material testing positive for an objectionable organism? This is significant to me and i believe Don as, in our experience, personal care production considers any Gram negative an objectionable organism.

A16: Would you mind offering the concepts involved in your "approve objectionable organism evaluation process?

A17: We initiate the report with the following information.

Details about sample, description, type of water, test type etc.
Determine if the isolate is commonly known to produce a biofilm and document the verdict.
Record the number of organisms recovered and indicate whether the organisms were recovered from enrichment and/or enumeration tests as applicable.
Describe Clinical Significance
Describe the isolates potential to spoil material
Evaluation of Potential Risk:

Reference:
Exclusion of Objectionable Microorganisms from Non-Sterile Pharmaceuticals, Medical Devices, and Cosmetics
PDA’s Technical Report No. 67

A18: I agree about the 100cfu/ml limit not being representative of what is being seen.
We also sample and filter 250ml samples and within those routinely obtain zero.

When i am talking about isolating Gram negative rods i am not trying to justify having 50cfu/ml, but rather an extremely low count i.e. 1 or 2 cfu within the full 250ml sample.

I'm also not convinced that the isolate is from the system and could also be a contaminant. However from what the current guidance allows (100cfu/ml, no specific statements for absence of all gram negative

bacteria, a known assumption/expectation of absence of specified organisms listed against the final product and the "dreaded" BCC), is it acceptable to have this type of situation, albeit extremely rarely and not what is happening in every sample to accept water with this contamination?

In my situation i am not trying to make this the norm, however plan for sporadic instances and have a plan documented that does not mean rejection of batches.

A19: I am trying to assess against what is defined within guidance in order to then (if required) be able to assess the isolated Gram negative and provide a robust justification for its acceptance. There is always the difficult discussion potential with an inspector and their opinion, however if we are complying with actual requirements shouldn't that be allowable.

The information i have to think it is justifiable is as follows.
100cfu/ml limit on PW with no statement to say that all Gram negative rods must be absent.
The specific Gram negative rod isolated is not defined on the finished product specification or is not Bcc.
It would be assessed and deemed as not objectionable in the view of the intended product. As assessing and defining and additional objectional organisms for a finished product above those specified, is performed.

From any 483's i have looked at they seem to say isolation of organism that may be objectionable in view of its potential usage and is usually because the manufacturer has just ignored its isolation and not performed any investigation or have any assessment when it happened.

I do believe that every effort should be taken to bring the system under full control and ideally have zero isolation. A more well-maintained system that is functioning in line with its original qualification the better not deteriorate causing more significant issues.

However, when this type of scenario does happen, as long as it is assessed and justified appropriately is it not acceptable?

A20: I agree with your comments. To make consistent science-based decisions about the potential for harm due to microorganisms in your materials and product, one needs to define “objectionable organism”. The FDA’s 1978 preamble to the GMPs says the following:

“Microorganisms could be objectionable by virtue of their total numbers or their detrimental effect on the product or by their potential for causing illness in the persons ingesting them…the objectionable nature of a microorganism may develop only in relation to the unique circumstances of a particular formulation, a particular ingredient, a particular method of manufacture, or the conditions found at a particular firm.”

Organisms isolated from a pharmaceutical water system, API, excipient, or container system should be evaluated to determine its objectionability as per 21 CFR 211.84 and 21 CFR 211.113, but not every Gram-negative bacillus will have a detrimental effect on the product or cause harm to the person ingesting them and therefore cannot, by the FDA’s own definition, be considered objectionable. I have no doubt that some investigators do consider any Gram-negative bacillus to be objectionable but that decision can’t be made without considering the product characteristics, route of administration and patient population. And if the water system must be free of all Gram-negative rods, then any API, excipient, or container system would have to be held to the same standard since all contribute to the bioburden of the product. Although I agree that having no Gram-negative bacilli in the water system eliminates the need for a risk assessment to determine if the organism is objectionable, the fact that it is a Gram-negative bacillus alone does not meet the regulatory definition for an objectionable organism.

Water Activity Qualification

I used to view the Water Activity (Aw) assay from a perspective similar to that applied to a pH assay. In other words, an IQ/OQ/PQ is performed on the instrument, a method is issued and controls are included every time the meter is used (and/or periodic performance against standards). More recently, I a client

asked to validate the assay using their product in a way similar to a microbial limit test. I tried to explain that our approach on the meter qualification which is not tied to any specific material but they are insisting that is not the approach they want but they also can't specific details as to how they want this done. I was then more confused when other clients asked similar questions. I though the questions came as a result of the recent changes in Aw USP chapters but a review of such chapters don't change my perspective. Can the forum members provide their input since I'm here feeling there is something I'm not seeing?

Micro lab - RTU - air circulation

Can someone please guide me on the RTU air circulation requirement for Microbiology Laboratory? We are thinking about having 20% fresh air and 80% circulating in the design.

Verification: Problem with TYMC on 35C

My colleague has a problem with verification of product (API: perindopril+amlodipine).

Test (pour plate method) gave these results:
- product diluted 1:100 gives the adequate result for TAMC growth;
- product diluted 1:10 gives adequate growth for TYMC on 25°C, but no growth on 35°C. (Positive control has growth on 25°C/35°C).

What should she do next?
In regular analysis we test TYMC on 25°C, but during the verification she has to test it on 25°C and 35°C.

A1: Why qualify TCYMC at 35 degree C when USP <61> specifies a 20-25 degree C incubation for < or = 5 days on SAB for method suitability?

A2: My mistake, I didn't understand her problem.

Problem is this:
Verification for TAMC on 25°C and 35°C.
In this test Aspergillus brasiliensis did not grow on 35°C (with sample diluted 1:100), and positive control did.
In the same test A.brasiliensis on 25°C had growth (sample diluted 1:100).

What to do in this situation?
What would cause this?

A3: You need to be more precise in stating the incubation temperatures. TAMC is conducted using soybean-casein digest agar incubated between 30-35 degree C so in practice the indication temperature is the middle of the range 32.5 degree C. Also what did you use as a positive control as none is recommended and there is no 25 degree C verification in USP <61>.

At temperatures above the optimum growth temperature the growth falls off rapidly. Maybe the temperature if it was above 35 degreeC was too high for the A. brasiliensis.

A4: Incubation temperature at 35.0C for Aspergillus brasiliensis may not be the reason as to why the culture did not grow at this temperature. Generally, the optimum growth temperature for the genus Aspergillus is typically greater than 30°C (Takatori, 1991). The optimum growth temperature of A. brasiliensis is 33.0°C with an upper limit of 42.0°C (Takatori, 1991) . Koide and Yosokawa (2008) reported that the growth rate of colonies and spores of A. brasiliensis increased as the culture temperature is raised to 30.0°C, after which the growth rate is maintained up to 35.0°C. In 2011, Alborch had reported that the optimal growth temperature for Aspergillus brasiliensis is 25 to 40.0C. If the incubation temperature was up to 40.0C, I do not think that it would have prevented the growth of the strain.

References: Takatori, K. （1991）Hitome de wakaru zusetsu kabi kensa・ sousa manual（in Japanese）. pp. 20-21. TECHNO SYSTEM, Tokyo.

Koide, S., and Yasokawa, D. （2008） Growth prediction of mycelial mat and fruiting zone diameters of Aspergillus niger subjected to temperature changes （in Japanese）. Nippon. Shokuhin Kagaku Kogaku Kaishi, 55（7） 338-344

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Furthermore, it was stated in the original post that a 1:100 dilution had to be performed for a TAMC with an incubation temperature at 35.0C and a 1:10 dilution can be performed for a TYMC with an incubation temperature of 25.0C. It seems that a lower dilution of the API test sample (perindopril + amlodipine) could not be accomplished for demonstrating bacterial recovery. It seems that this API is a blood pressure medication which I suspect would not be susceptible to microbial contamination in the first place based upon each of the chemical manufacturing processes that are used to produce each of the components of this API (perindopril and amlodipine).

A5: I read your answers regarding the question of Amira Ranica about As. brasiliensis growth.

Aspergillosis is the human disease which is caused by the Aspergillus species but rarely caused by the As. brasileinsis, but it can infect the humen, and the temperature of human body is 37 °C. It shows As. brasiliensis can be grown at little higher than its optimum temperature.

First of all, according her question, TAMC is not for the total yeast mold count. It might be possible that she treated it as TAMC whereas rest of the sample including positive control treated as TYMC.
Second, might be possible she used soybean-casein digest agar instead of sabouraud dextrose agar and incubated at 35 °C, soybean-casein digest agar can also support molds and yeasts but less than the dextrose agar, whereas positive control she treated at dextrose agar and incubated at 25 °C.
Third, microbiology is totally depended upon the probabilities, it is possible that the first dilution hadn’t such numbers but the second dilution of the same sample had much numbers to grow.

I could be wrong but above mentioned things could be occurred.

VRBA v. VRBGA and TGO 100 Requirements

The lab I work in currently follows USP methods for Coliform analysis using Violet Red Bile Agar (VRBA) and it contains lactose. On the other hand since we are audited by the Therapeutic Goods Administration they require in TGO 100 that coliform analysis should be done using Violet Red Bile Glucose Agar (VRBGA).

I understand that Lactose is made up of Glucose and Galactose but is that sufficient justification for the TGA to accept the USP method?

A1: USP <62> requires the use of Violet Red Bile Agar (VRBA) containing glucose for the test for absence of E. coli in non-sterile drug products not for coliform analysis.

A2: VRBGA is designed for Enterobacteriaceae recovery. Not aware it is effective in differentiating coliforms unlike VRBA with the pivotal lactose ingredient. Does TGO 100 specify subsequent testing?

A3: I neglected to mention that our analysis is for nonsterile nutritional and dietary supplements. As such USP <2023> Bile Tolerant Gram Negative Bacteria are what we are testing for.

My predecessors set up the testing for coliforms using Chapter 4 of BAM. I've been tasked with aligning testing to TGO 100 to our current analysis.

Just to answer some questions:

USP <62> requires the use of Violet Red Bile Agar (VRBA) containing glucose for the test for absence of E. coli in non-sterile drug products not for coliform analysis.
So under <62> testing for Bile-Tolerant Gram-Negative we should have been using EE Mossel Broth and VRBGA. FDA Chapter 4 BAM states for coliforms on solid medium can be done on VRBA.
VRBGA is designed for Enterobacteriaceae recovery. Not aware it is effective in differentiating coliforms unlike VRBA with the pivotal lactose ingredient. Does TGO 100 specify subsequent testing?
No, TGO 100 only states that Bile-tolerant Gram negative Bacteria should be tested for.

I suppose I answered my own question but was wondering if anyone here had experience with using FDA BAM in place of TGA requirements under TGO 100.

<81> ANTIBIOTICS—MICROBIAL ASSAYS

Currently we are trying to assemble and develop an analytic method for two products, APIs Vancomycin and Colistin:
- How would you recommend starting the process?
- Has anyone had any troubles working with *Bordetella bronchiseptica*?
- If specific Antibiotic media aren't available at the moment, how could you establish similar ones to use?
- As per <81> USP says, the selected methodology is 5 x 1 (5 Std vx 1 sample), have you successfully applied this method? And the 3 x 3 method?
- Is there a fast efficient way to process all the data?

BTGNB versus test for test for coliforms and enterobacteriaceae

Can someone please explain the difference between testing for Bile tolerant Gram-negative bacteria and test for coliform and/or enterobacteriaceae? I see COA's from raw material manufacturers that test for this, and am wondering what is the difference, if any.
I have scoured the Internet, recently and in years past, and find no clear explanation. If someone is testing for BTGNB, is that the same as testing for Coliforms and Enterobacter?

A1: I also see "Absence of Enterobacteria" occasionally on COAs and have explored this a bit. It likely comes from the pre-harmonized compendia before ~2008. A little history: prior to harmonization, Microbial Limits enumeration and absence tests were all bundled under general chapter USP <61> and the USP chapter did not have an absence test for "Enterobacteria". However, the pre-harmonized European Pharmacopoeia chapter 2.6.13 did have a test for Enterobacteria. It was titled "Enterobacteria and certain other gram-negative bacteria" and used lactose broth, Mossel broth, and VRBGA for detection. When USP and Ph.Eur. harmonized, USP <62> was born and the "Enterobacteria" test from Ph.Eur. was adopted with a few changes such as replacing lactose broth with TSB, replacing 36-37°C incubation with 30-35°C, and change in title to "Bile-Tolerant Gram-Negative Bacteria".

My best guess is your vendor specification was set prior to harmonization and the vendor has not kept up with compendial harmonization terminology.

That's my take, but I could be wrong. I have not confirmed any of this, however when requested to add a test for "Absence of Enterobacteria" by FDA to a material specification I have added the BTGNB test and it has been accepted.

Grade B microbial identifications

I thought I had read that the EU was requiring microbial identifications of all Grade B recoveries (regardless of count) but cannot find the reference. Does anyone else recall this and do you have reference to it? It may have been proposed but never instituted.

A1: I would recommend looking into the Annex 1.The second targeted stakeholders' consultation was published February 20, 2020 and is currently in circulation.

[cid:image001.png@01D84F64.A2202850]

You can download the guideline under consultation here: https://ec.europa.eu/health/other-pages/basic-page/second-targeted-stakeholders-consultation-revision-annex-1-manufacturing-sterile-medicinal-products_en

A2: Hi Dina. Yes, the new EU GMP Annex 1 document that's still in draft form explicitly states that you'll need to identify and risk assess any isolates from Grade A/B. It also states you should consider identification for alert and/or action level excursions.

A3: Here you have the statement for the Draft 12 of the Annex 1 point 9.31

[image: image.png]

Everyone expects to have the document approved at the end of this year so that will be in force right in that moment

The same applies to any micro detected in WIFI samples, bioburden or any other component in direct contact with the final product.

Allowable time between sub culturing <61> <62>

How can i go about defining the timeframe from when a broth is removed from the incubator that the subsequent subculturing and incubation must be performed. e.g. from when EEB is removed to when VRB is incubated or RV is removed and XLD incubated. my question is related to all stages where any item is removed to the next stage being incubated.

During method suitability we did not build in any timeframe assessment or never thought to. However in routine we can see these types of activities take anything from 5 minutes to over an hour as we may be trying to align multiple different tests to maximise the testing and operators' time.

What could the possible impacts be if this activity was not done for up to several hours?

If i do need to perform some retrospective work to cover this what could i do?
Must it be during method suitability with product or could i perform each enrichment test route with inoculated organism and assess different 'stand times' before subculturing and then assess that growth is obtained?

USP 61 suitability testing

I have a question on a USP 61 suitability test that I recently encountered. I'm hoping that the forum might be able to provide a little insight.

I have a suitability test for USP 61 in which the product was diluted in TSB to a 1:100 dilution. From this dilution, replicates of 1 mL were pour plated for each challenge organism. This gives the test a sensitivity of <100 CFU/g. However, in a routine test that claims to be following this suitability, I found replicates of

10 mL being plated in an effort to achieve a sensitivity of <10 CFU/g. I'm having trouble accepting that this is a justifiable practice, as the specific method validated during the suitability test is not being followed. Is it acceptable to adjust plating volumes from what was established in a suitability test? What would be the impact of this practice? I would expect that plating 10 mL of the sample dilution would introduce more of the sample than 1 mL would, and create a risk of addition inhibition. Am I on the right track?

A1: Testing of 10 mL when suitability is performed with 1mL may not be acceptable to regulatory inspector and may write an observation. Also, I believe 10 mL is not acceptable plating volume.

I had one product containing 10CFU/g limit but needed to dilute to 1:100 to overcome the inhibition, and lab proposed to plate 1 mL in 10 separate plate to report the sum of 10 mL test of 1:100 dilution and to report <10CFU/g. The risk doing this is any colony observed is OOS. Luckily so far we have not observed any count though.

A2: Plating a volume greater than 1 mL used in the method suitability test would not comply with the method you qualified. This could affect the organism recovery due to inhibition and even dilute the medium at higher volumes.

A3: You are on the right track.
The testing you described is not in compliance with the method that was qualified.
Suitability must be performed to support the current test method.

A4: Assume the 1 ml plated ten times was from a collected 10 ml volume. Otherwise these would be 1 ml replicates.

A5: The method asks to take 10x1mL to plate from the dilution 1:100 that was found suitable for 1mL plating. The sum of the 10 plates CFU would give you the results per g of the product being tested.

A6: The sentence, “I found replicates of 10 mL being plated in an effort to achieve a sensitivity of <10 CFU/g,” needs clarification.

Does this mean ten 1-mL samples were being plated? Or something else? If the former, this appears to be ten replicates at a 1:100 dilution, which in most cases, would be averaged and multiplied by 100 to get the count.

If you need a 1:10 maximum dilution, and you cannot achieve this, then other methods to neutralize the test sample should be considered, including membrane filtration, and adding additional neutralizers to the dilution tubes and/or agar medium.

A7: We have one Antibiotic API, need to the method suitability for Microbial enumeration test and test for specified microorganism. We tried at 1:100 dilution by using Soya lecithin and Polysorbate 80, but bacterial recovery is nor gain for enumeration as well as Specified microorganism. However fungal recovery obtained.
material in insoluble in water so Filtration is not possible.
I check the vendors method he used 5-micron membrane filter then used the filtrate for further test.
Vendor used filtration method. As per USP NMT 0.45 micron membrane is used for filtration method.

Is it ok to use 5 micron membrane for microbial test?

I had performed method suitability by Pour plate and taken two different diluent for two trials, results of both trials are same i.e. No recovery of bacteria in either enumeration as well as specified microorganism. Can I conclude as per the USP clause that failure to isolate the inoculated organisms is attributable to the microbicidal activity of product and analysed the API as per my final trial of method suitability.

A8: You can take one more trial to add the soya lecithin 0.5 and polysorbate 80 1%.
In the SCDM media as well as in SCDA media.

So your neutralisation level will be enhance during reconstitution and incubation condition at both dilution 1:10 and 1:100.

And check most effective method
If no recovery then can conclude with refrance of USP chapter.

A9: In reviewing your post concerning method suitability on an insoluble antibiotic API, I'm a little confused. For example, it is well known that Soy Lecithin and Polysorbate 80 will generally not be able to neutralize the antimicrobial activity of most antibiotic API's. However, there are some chemical ingredients that can be used to inactivate the antimicrobial activity of an antibiotic, but it is hard to recommend one without knowing the identification of the antibiotic. Furthermore, you state that the tested antibiotic API is insoluble in water and the supplier uses a 5-micron membrane filter to filter a solution of the insoluble antibiotic and the filtrate is tested for microbial content. This step does not make any sense. From my perspective, the 5-micron membrane filter will not be able to remove any microorganisms from the test sample. I can see the 5-micron membrane filter would be able to remove undissolved particles of the insoluble antibiotic in water that results in an aqueous filtrate. It seems that something is missing here. Is the supplier pre-dissolving the insoluble antibiotic API in a non-aqueous ingredient such as Isopropyl myristate or some other chemical which is indicated on the MSDS of this antibiotic that can be used to solubilize this ingredient? I see that you have not been able to recover bacteria suitability test isolates in both the enumeration and specified microorganisms' portions of a microbial limits test. I suspect that the resulting filtrate is not aqueous and there is the presence of the antibiotic API in this filtrate.

Furthermore, I do not understand why you are performing a microbial enumeration and specified organism testing on an antibiotic API in the first place. I would think that a conducted risk assessment would have determined that this insoluble antibiotic API would be considered as a hostile raw ingredient to bacterial organisms and is not susceptible to bacterial contamination.

Media Growth Promotion Sterility Checks

What is the current guidance on the number of containers or plates to be used for sterility checks on commercially prepared media during growth promotion testing?

A1: USP <71> states: Incubate portions of the media for 14 days. No growth of microorganisms occurs.

It is not prescriptive on purpose. In practice with purchased media the vendor incubates the entire batch prior to inspection and would exclude non-sterile units, low fills, damaged containers and other cosmetic defects. On receipt you incubate a portion of the incoming lot and inspect each unit at the time of conducting the test. You need to use good judgement.

Negative or manipulation controls are used in the test and they are available to compare the test containers against.

A2: There is no such requirements for total no. Of plates to perform sterility test since the each lot is subjected for pre incubation before usages.

Generally in the industry sterility testing is performed by using one pack/wrap i.e. 5-10 sterile plates on annual basis.

A3: While I agree with [name redacted] that a sleeve (e.g., pack/wrap) is typically used, we always tested per lot per shipment rather than once per year. It was for a sterile manufacturing site so the risk to a contaminated plate being exposed in the MFG environment is greater than if you are in non-sterile. Either way, in my experience material release is typically based on per lot per shipment.

A4: It's expected that each shipment, regardless of being the same lot, be growth promoted due to shipping variabilities. Firms usually stick one sleeve, unopened in the incubator for sterility check and use another sleeve for GP, possible even a few different sleeves to show consistency. There is no exact

number of plates that need to be tested. The only guidance is that each shipment and/or each lot be tested with GP.

preservative efficacy testing

Hi All, i am looking at the differences between EP and USP testing. i know that there are different time periods in which testing needs to be performed however i am looking to see what the interpretation of a specific part in the EP is.

Remove a suitable sample from each container, typically 1 mL or 1 g, at zero hour and at appropriate intervals according to the type of the product and determine the number of viable micro-organisms by plate count or membrane filtration.

Within the acceptance criteria tables there is no Zero hour information. The earliest is a 6 hr within one of the criteria.
Does the statement above (for zero hour) require you to perform the test procedure immediately and if you do what do you do with the results as there is no expected reduction?

We have interpreted it as a Day 0/time zero and that the testing must be carried out immediately. The next test time for our product is 2d so we have not really thought that we were testing at day/time zero incorrectly and maybe it isn't required. But im now thinking that if you have a 6hr would you really be expected to do a zero hr also!.

A1: It's pointless. Efficacy is determined " against the value obtained for theinoculum" determined as "the number of colony-forming units per millilitrein each suspension by plate count or membrane filtration". And I would not be so confident of no reduction.

A2: I do agree with Phil’s opinion that performing a microbial count immediately after inoculation of a preservative challenge test sample is useless. You are correct that Ph. Eur. 2.1.3 states that a suitable sample be removed from each container, typically 1 mL or 1 g, at zero hour and at appropriate intervals according to the type of the product and determine the number of viable microorganisms by plate count or membrane filtration. In conclusion, it is a requirement that Zero-hour counts be performed immediately after inoculation if a E.P. challenge test is conducted for a test sample.

You are correct that the European Pharmacopoeia challenge test does not list log reductions for the Zero-hour time point for various tested types of products. There is no indication in the chapter as to why to perform a Zero-hour count for a test sample. Why conduct it in the first place?

I would not be surprised that the designers of this method thought that by incorporating a Zero-hour count immediately after inoculation would provide evidence that the sample had been inoculated at the proper level. However, this is not true because there are certain types of preservatives that will immediately kill challenge test organisms upon inoculation of a product formulation.

This is probably one of the reasons as to why E.P and U.S.P. challenge test methods have not been harmonized with each other and it looks like that they will never be harmonized. In looking at the challenge test criteria in Tables 5.1.3.1 and 5.1.3.2 of the European Pharmacopoeia 5.1.3 Efficacy of Antimicrobial Protection, I do like the more stringent challenge test A criteria in comparison to the USP for the indicated products in these tables. I have never been a fan of the B criteria in the E.P. challenge test because I just feel that it leads to confusion concerning preservative adequacy of a formulation. For the products in Table 5.1.3.3, I feel from my perspective that it would be more appropriate for these products to have the same challenge test criteria as the products listed in Table 5.1.3.2.

A3: I agree that performing at time zero does not provide any information that is useful or needs to be assessed within the criteria.
Also, at time zero we are actually seeing significant count reduction to the product inoculum count (3-5 log reductions) immediately.

I was just hoping that I was mis-interpreting and doing this in error to try and remove. But as it is stated then I think we must still perform for EP.

A4: I agree with [name redacted] interpretation of the zero time inoculum recovery. I have seen samples in which there was no recovery, or very low recovery at this sample time. I remember the first time that it happened, I just assumed that I had missed the inoculation.

Method suitability - failure to inoculate with correct expected count

When performing method suitability testing and you are required to inoculate with a specific number of organisms e.g. <100cfu,
If your count plates achieve greater than the required inoculum e.g. 120cfu, how do you document this and initiate a repeat?
We currently raise a deviation to the protocol, where by we need to describe what happened, why and any impact. then identify corrective action and execute the repeat and then report the results.
This feels like an un-necessary exercise as all we are saying is that we didn't get the inoculum count to the required value in order for the test to be considered valid and therefore any results to be assessed.
In an analytical test this would be treated as a failed system suitability and results would not be usable.

What approach do you use?
Is it appropriate to just have an approved up front statement that says if the counts are not within limits then the results are not able to be used and the test can be repeated?

If we do not meet 50-200% then we would raise a deviation to investigate and document our rationale etc for repeat. These are mostly due to counts being very low (e.g. <15) and the resulting statistical variability from trying to compare very low-level results.

A1: Yes, your procedure should treat this as a system suitability failure. Invalid test, repeat, retain documentation. It is a best practice to track/trend invalid results in the lab.

A2: If we see >100 CFU per inoculum from suitability positive control, we are invalidating the suitability study, we also mention this in protocol that the suitability can be repeated if invalid results obtained. If >100 CFU from the plate with sample observed and recovery ratio is within factor of 2 the suitability will meet the USP <61> criteria.

A3: Hi Michael. Your example is a little confusing. If your control plate of <100 cfu is 120, that's normal as you stated, within the "factor of 2". But then you're asking about lower counts in samples...which I assume is not within the 70% recovery as stated in USP 1227 . If your case is that your control plate is 120 and your sample plate is 15, that's a log reduction and would fail. Even if the control inoculum was 80, that result would still fail.

I think some clarification may be needed.

A4: Thank you for replying to gather more information and try and help me. I just re-read my information and can see how it may not have been interpreted how I intended.

There are two different examples

1. Having the positive control counts above 100cfu and therefore not able to meet the expectation of a low level inoculum (<100cfu). So the test is not valid to allow assessment.
2. From your reply, I realise that there could be >100cfu in the positive control but approximately 50cfu in the test. This could be an issue with neutralisation efficacy, however in the first instance I think I should still invalidate due to failing to inoculate with the required low level count. The repeat may show a %recovery failure, but I would then record this as a deviation failure and investigate.

1. Not meeting the 50-200% was really additional information where i was saying that in this case i would raise a deviation to investigate (for example, this could be an ineffective neutralisation and method would need adjusting). However, these failures in my experience are from having very low counts on both the test and control plates and therefore smaller count differences have a larger impact on the comparison.

Final thing regarding the 70% recovery. My understanding is that this is for assessing an alternative method and 50-200% is used for method suitability of the compendial methods. I may need to check this again.

A5: Thank you for the clarification. Yes, those are failures. Now I understand when you're talking about lower recoveries. I'm not sure there's much you can do about a lower CFU due to variability within lyophilized/pelleted organisms other than try different vendors unless you're performing the dilutions yourself.

Yes, I quoted USP 1227 and I'm guilty because I should know better....it's been a cause of confusion for many years! USP 61 states a factor of 2 and I should've clarified that.

B. Diminuta

What type of agar do you use to reactivate B. Diminuta?

I know that I can reactivate it in nutrient agar but I would like to know your opinion, since after reactivating it I must standardize it to be used in the filter efficiency test.

A1: I believe the ASTM standard method requires cultivation in saline lactose broth on a rotary shaker to standardize the B. dimuta size for sterilizinfg filter validation.

Interpretation of AET results

When performing the subsequent timepoint tests I am trying to define which dilution should be used for calculating the count of cfu/ml.
I believe that for method suitability we should be using results of 25-250 for bacteria+yeast and 8-80 for Aspergillus. Is this the case for assessing the obtained counts during actual testing or do you use the highest dilution that has any counts no matter how low?
For example
If you obtained the following results for an organism how should you interpret?
10-1
10-2
10-3
31
34
8
4
0
1
Do you use the 10-1 as this has provided counts in the recommended countable range or should you use 10-3 as this would be a worst?

10-1
10-2
10-3
90
94
20

21
0
0
In this example would you use the 10-1 as this has the higher counts or 10-2 as the counts are just below the range but again are a worst case.

A1: It is my recommendation that you should use the same dilutions for the suitability counts if possible for bacteria, yeast (25 to 250) and Aspergillus (8 to 80 CFU) for the time points of a challenge test. In most cases with different preservative systems, it should be a 1:10 dilution for the most part, but sometimes a 1:100 dilution would be required to obtain counts for a challenge test. The only times in which higher dilutions will be need it is when there is a definite challenge test failure. For this reason, I like to obtain counts before the 7-day timepoint in order to adjust the dilution levels for the other timepoints.

Detect (1→3)-ß-D-Glucan

These two method (BET Chromogenic method or BET turbidimetric method) which method is more sensitive to detect (1→3)-ß-D-Glucan? In antibiotic products. Please share.

A1: This one sounds easy but it is really quite complicated. The quick answer is neither or both. It is entirely driven by the manufacturer and the lysate reconstitution buffer. The extraction process by which the raw crab blood is processed differs, fairly substantially, between the three major US suppliers (Associates of Cape Code, Lonza and Charles River) and that largely drives the differences in reactivity. These are proprietary processes so I can't go into them in detail but if you search the internet you can find the information if you really need it.

Associates of Cape Cod controls Beta Glucan reactions in their lysates by using a blocking buffer at reconstitution. It works quite well but it slows the lysate reaction just bit. It is easy to use as you just reconstitute the lysate with it. It can be used with most of their Tubidimetric or Chromogenic lysates.

ACC has recently released a recombinant assay for BET. The lysate has no Beta Glucan pathway. It has no reaction to Beta Glucans. It is a full cascade recombinant so it utilizes a chromogenic reaction that is easily adopted if you are already using either turbidimetric or chromogenic assays.

If you are trying to measure Beta Glucan Associates of Cape Code manufactures a detection kit for that.

Lonza, also uses a Beta Glucan blocking buffer but they use it as a sample pretreatment making it slightly more complicated to use, in my opinion. It can also be used with most of their lysates if I'm recalling correctly. Without blocking the sample with buffer Lonza KQCL, is largely unreactive to Beta Glucans, but it varies from lysate lot to lot and sometimes still requires blocking buffers for some samples. I'm a little fuzzy on their Tubidimetric method but it would have some degree of Beta Glucan reactivity and some samples would likely have to be blocked.

Charles River Labs I'm not as familiar with but they would also have some reaction to the G pathway and I'm sure they have a method to block it. I'm fairly certain both their Chrome and Turb methods have some degree of reaction but I'm not sure which one would be less reactive.

Acceptance criteria for microbiological quality- Interpretation of the results

Interpretation of the results

I have doubt regarding interpretation of results in the microbial contamination test. the query is as follows

The total aerobic viable count (TAC) is considered to be equal to the number of CFU found on Casein soyabean digest agar. If colonies of fungi are detected on this medium, they are counted as part of TAC.

The total fungal count (TFC) is considered to be equal to the number of CFU found using Sabouraud dextrose agar with antibiotic.

Acceptance criteria for microbiological quality should be interpreted as follows:

10*1* CFU : maximum acceptable count 20

10*2* CFU : maximum acceptable count 200

10*3* CFU : maximum acceptable count 2000, and so forth…

What is the meaning of this acceptance criteria? If the manufacturer decides the limit of non sterile products is not more than 1000 cfu per g and we get 1500 cfu per g results. In this case samples will be complies or not complies. According to acceptance criteria 10*3* CFU : maximum acceptable count 2000 this sample should be passed.

Where applicable this acceptance criteria and what is the meaning of this acceptance criteria.

A1: You may have noticed the USP cites the limit as NMT 10^3 CFU/g with the maximum acceptable count as 2000 cfu/g.

I alway felt If you set your specification as NMT 1000 CFU/g you have locked yourself in to not exceeding 1000 CFU/g.

A2: I expect some will disagree, but here goes.
Acceptance criteria of 10*3* CFU per g and not more than 1000 CFU per g are not equivalent.
If your acceptance criteria is not more than 1000 CFU a result of 1500 CFU fails.
If your acceptance criteria is 10*3* CFU a result of 1500 CFU passes.
Using the acceptance criteria of 10*n* CFU takes into account the lack of precision in microbiological testing.
It is applicable when performing testing to a compendia that accepts using the log value rather than a more precise numerical value.

A3: If acceptance criteria is 1000 cfu, then your sample does not complies. If acceptance criteria is mentioned as 10*3, then your sample is pass. Please go through USP <1111> for more information on acceptance criteria.

In use actuation study - non-sterile nasal spray

Has anybody heard of or have any reason of why you would perform this type of study.

For a non-sterile nasal spray we have been asked by a customer to perform actuations of units (every other day) for up to 1 or 2 months, depending on the starting fill volume.
Following the actuations, a sufficient number of actuated containers are then tested for AET. In order to test for AET we must pool the actuated units to allow the initial starting fill volume to be re-made (as product would have been utilised during actuation) to allow the AET to be performed.

What is the possible reason that this has been requested and is it a requirement from any country or document?

I'm assuming that they are trying to show that somehow in use that the preservative is still effective. It isn't trying to recreate accidental contamination in real life as its not subject to being put up a nose and isn't that the whole point in the AET test to artificially challenge with variety of organisms.

Could they be trying to assess if the amount of preservative is equally and evenly distributed during use is still effective and will pass AET.

Just for additional information, they are also doing the in-use study for the analytical tests.

A1: Perhaps relevant to EU's silly PAO (period after opening) labeling.

A2: To add to this conversation, Period After Opening labeling is only used in the EU in which a cosmetic product has a shelf-life greater than 30-months but has no expiration date. I believe that all drug products would have an expiration date instead of having a label with a Period After Opening. I agree with Phil that a product label with Period After Opening is silly because it really doesn't mean a thing. An expiration date for a product could be up to 3-years while a Period After Opening label for a product can be generally up to one year. Generally, expiration date for a product is set by performing product stability studies (e.g., analytical, and microbial studies). However, it is my understanding that there is no agreement upon standard test methodology within the cosmetic industry in how a Period After Opening should be set for a product formulation. Companies will have different PAO labels for the same product categories.

Regarding the in-use actuation study for a nasal spray, the only thing I can think of is to determine whether the preservative system is compatible with the composition of the various components of the actuator system. Instead of performing this type of study, I would think that it would be easier to do a literature search concerning the compatibility of chemical composition of actuator parts with different types of preservatives. In addition to performing an AET on unused product containers, I would also recommend that a clinical in-use study be conducted on consumer used nasal products and test each used container for microbial content to see how well the preservative system in the product worked in controlling microbial contamination in real-world conditions.

A3: i have identified the following in ICH Q8(R2),

Although chemical testing for preservative content is the attribute normally included in the drug product specification, antimicrobial preservative effectiveness should be demonstrated during development. The lowest specified concentration of antimicrobial preservative should be demonstrated to be effective in controlling micro- organisms by using an antimicrobial preservative effectiveness test. The concentration used should be justified in terms of efficacy and safety, such that the minimum concentration of preservative that gives the required level of efficacy throughout the intended shelf life of the product is used. Where relevant, microbial challenge testing under testing conditions that, as far as possible, simulate patient use should be performed during development and documented in this section.

Maybe this is similar to what you mention Don with clinical used samples rather than an actuation study performed within the lab and in a clean environment (Class II safety Cabinet).

Thoughts anyone?

Personnel Monitoring

We perform environmental monitoring on personnel in our Grade A cleanroom.
The sampling points are the chest, forearms, and finger dabs.

I would like to enquire where on the forearm is a contact plate placed?
Is it placed between the wrist and elbow (mid-point) or the inside of the elbow.

Operators wear long length gloves during their activities.

Any assistance will be greatly appreciated.

A1: The location where to sample the forearm depends on the type activities performed / interventions made by personnel in combination how and where these are performed in the process. This should be written down in a risk assessment from which the worst-case sample location is then to be determined.

Microbial Contamination Test

I have a general query on microbial contamination.

TAMC-Total Aerobic Microbial Count (Bacteria + Fungi); TAC-Total Aerobic Count (Bacteria + Fungi); TAVC-Total Aerobic Viable Count (Bacteria + Fungi) and TPC-Total Plate Count (Bacteria + Fungi) are same or different i.e same meaning. Are these synonyms? Similarly, are TFC- Total fungal count (Yeast + Mould) and TYMC- Total Yeast and Mould Count are same?

In analysis of microbial contamination in non sterile dosage forms, we perform TAMC and TYMC. In the case of TAMC, the general limit is NMT 1000 CFU per g. This limit is only for bacterial count or for both i.e Bacteria and Fungus. Because in pharmacopoeia it is clearly mentioned if colonies of fungus are detected on SCDA media then it will be counted as part of TAMC. If the limit is for both then what is the necessity of TYMC limit i.e in general 100 CFUper g. If fungus is detected on SCDA media then why is it a separate requirement of the TYMC test? Can we assume that for TAMC, 900 colonies of bacteria and 100 colonies of fungi (total 1000 cfu per g) so that we can easily identify and interpret results?

Sometimes it is possible that there are no fungal colonies found on SCDA plate while found on SDA Plate. In this case we consider TAMC as Total Bacterial Count for SCDA plate.

Some manufacturers perform TBC-Total Bacterial Count (Limit is NMT 1000 CFU/g) and TFC-Total Fungal Count Test (Limit is NMT 100 CFU/g) for drug products. In all Pharmacopoeias the method is the same as for TAMC. In this case if we found 50 colonies of bacteria and 20 colonies of fungus on SCDA plate then how we interpret results for TBC. We report results only 50 colonies or 70 colonies for TBC? For TFC if 25 fungus colonies are detected on SDA media, then we consider the result 25 colonies or 45 (20+ 25) colonies.

Is TBC and TAMC the same?

Please consider above conditions and suggests.

A1: The media and incubation conditions are defined for TAMC and TCYMC in USP<61> so you count all the colonies on the plate and based the dilution report the result as CFU/g or mL.

The exception is if bacterial colonies predominate on the SDA plates so that you may exceed the 100 CFU/g or mL limit then add an antibiotic to suppress the growth of bacteria, usually members of the genus Bacillus that can grow at low pH and high carbohydrate levels.

USP <61> uses the terminology TAMC and TCYMC.

EM Sampling of Medical Grade O2

What is the industry standard for sampling medical grade O2 that is piped into bioreactors? What equipment do you use to sample nonviable particulates and viable air? Any insight is appreciated!

API Drug Substance Vendor MLT Specifications

We are currently adding a new drug substance API and have sourced two qualified vendors for the material that will be used- one will be primary and one is for backup. The vendor certificates have micro specifications, but being different vendors they are performing very different tests for specified

microorganisms. I've been asked to pick and choose which micro specifications to adopt for our own internal release requirements.

My experience with vendor micro testing is they tend to attempt to cover all possible customers' requirements. One vendor, for example, seems to be testing nearly all specified microorganism tests while the other only does E. coli.

The API does not have a USP monograph, although there is a draft routing in PF. The draft monograph does not list any MLT specifications. The API is naturally derived from a plant-based source so it makes sense to have some level of incoming micro testing. It will be used to make an oral product that will have TAMC, TYMC, E. coli, and BCC testing.

If you add micro testing for a raw material and that testing is not directed by a monograph, do you typically adopt limits based on the final product? It might make sense to test API for E. coli, but testing BCC doesn't necessarily make sense especially when a vendor isn't doing that test themselves.

Your thoughts and experiences?

A1: Is the API being used in a solid oral dosage form, e.g., compressed tablet or a liquid oral dosage form, e.g., a syrup.

Based on the API manufacturing process, mother liquor, physical attributes like water activity , and testing history, you could justify after a risk assessment to not have a microbial specification or have a TAMC, TCYMC, and absence of E. coli. A although it is unlikely that the API will contain Bcc you could add it for a liquid oral dosage form but that would be a conservative decision.

A2: The product needs to be tested for all 4 microorganisms and Burkholderia. Go to ICH guidance Diagrams 6 and 8 . you will get some idea.

A3: The API is for an oral liquid finished product. Since it's a newly added API to our product portfolio I don't have any test history or other details such as Aw at this time. Right now it's exclusively a paper exercise based on what I've been handed (vendor CoA's), and a request to figure it out.

I must say, I'm a bit reluctant to add micro specifications on a material for a test the vendor does not also perform. From past experience, a failure due to a tighter internal specification or an internal specification the vendor does not include is a toss-up whether the vendor will take action versus tell you to pound sand when there isn't clear guidance on requirements, such as a monograph.

A4: For API Microbiological Testing we had ICH Q6A Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and Drug Products Chemical Substances. You can refer guidance document for API microbial Testing.

"Microbial limits: There may be a need to specify the total count of aerobic microorganisms, the total count of yeasts and molds, and the absence of specific objectionable bacteria (e.g., Staphylococcus aureus, Escherichia coli, Salmonella, Pseudomonas aeruginosa).

Please go through the document and set your specification.

A5: About BCC check with following link”

https://www.fda.gov/drugs/drug-safety-and-availability/fda-advises-drug-manufacturers-burkholderia-cepacia-complex-poses-contamination-risk-non-sterile

FDA advises drug manufacturers that Burkholderia cepacia complex poses a contamination risk in non-sterile, water-based drug products

<https://www.facebook.com/sharer/sharer.php?u=https://www.fda.gov%2Fdrugs%2Fdrug-safety-and-availability%2Ffda-advises-drug-manufacturers-burkholderia-cepacia-complex-poses-contamination-risk-non-sterile>

A6: From my personal experience working on numerous BCC-related contamination events over the past two years, there are raw materials that can be contaminated which might impact your nonsterile oral liquid dosage form.

Therefore, a risk assessment should be performed to determine what raw materials (including water) should be routinely tested to ensure BCC is not being introduced into the process and product. This may include an understanding of the process the raw material/API undergoes. Only then would you be in a position to determine what requires micro specs and at what level (numbers or absence, etc.).

As for vendors, many suppliers don't care what is in their material as long as it sells and the quality checks are passed on to their customers. If they should test for contaminants and refuse to do so, you may want to look at other suppliers.

A7: I assume this is a generic drug and other customers for the API have no microbial specification based on the synthesis, API recovery. and attributes.

If you have a specification and the vendor does not, if you reject a batch you may eat the cost.

A8: I would like to add some important considerations to this conversation for the setting of microbial test specifications for an API.

Before using ICH Decision Tree #6 to set or not to set microbial test specifications for an API, I think that it is important to determine whether the API will support or not to support the growth of microorganisms on actual production lots of the API. Before even conducting this testing, suitability testing will need to be performed as the first step of this process to determine if you are going to be able to recover the presence or absence of microorganisms. Granted, API's obtained from plants will often need to have the presence of microbial test specifications because it is common for botanical ingredients to be contaminated with microorganisms. In addition, it would be important to know the manufacturing steps of the API as to whether either hot temperatures or hostile chemical solvents are used in the manufacturing of an API from the plant material that might render it to be free from microbial contamination. In conclusion, a proper risk assessment will need to be conducted to determine whether or not to set microbial test specifications for a raw ingredient.

Furthermore, I find it to be common for different vendors of an API to have different microbial test specifications from each other especially if microbial test specifications are not present in the monograph for an API. Because of this, it is not unusual for a company to set their own internal microbial test specifications for an API or raw ingredient that is different in the microbial test specifications of the supplier. If this happens, this problem will often come up when the user of the material will reject it based upon their internal microbial test specifications and the supplier will say that the material is acceptable due to it meeting their own internal microbial test specifications.

BTGNB MPN method suitability

We are a non-sterile facility and perform micro limits tests on OTC as well as nutritionals. For nutritional BTGNB, the method is quantitative. I am curious as to how other labs perform their method suitability for BTGNB. I have two questions - one about method suitability and one general:

* At what point in the procedure do you inoculate with <100 CFU? (product suspension or EEB?)
* Do you perform serial dilutions from the TSB product suspension or separate dilutions?

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For example, we take 10 mL of product and add that to 90 mL TSB with tween and lecithin. Do you inoculate this product suspension with <100 cfu at this point, prior to the 2-5 hour resuscitation incubation at 25 degrees and prior to subculturing into EEB? Or do you inoculate each EEB tube containing product suspension with <100 CFU of organism?

10 mL product into 90 mL TSB (=product suspension = PS)

Then: (NOT serial dilutions)

* 1 mL of PS into 9 mL EEB (to give an equivalent of 0.1 g product)
* 0.1 mL of PS into 9.9 mL EEB (equivalent to 0.01 g product)
* 0.01 mL of PS into 9.99 mL EEB (equivalent to 0.001 g product)

I would like to know how other labs approach this as USP can be somewhat vague.

A1: Although, there isn't an official FAQ for <2021>, there is an official FAQ for your question about which BTGNB medium is spiked for UPS <62>. If you search "USP 62 FAQ" should be able to find it. The question you are looking for is #14. Here is the text:

“Q14”: What is meant by "at the time of mixing"? Bile-tolerant gram-negative bacteria: At the time of sample preparation, or at the time of addition to the resuscitation broth, or at the time of inoculation of the Mossel Broth? E.coli: At the time of sample preparation, or at the time of addition to pre- broth, or at the time of inoculation of the MacConkey broth etc.?

“A14”: In both cases, the micro-organisms should be added at the time of mixing with the preincubation or resuscitation broth. If Bile-tolerant gram-negative bacteria are taken as an example it refers to the sub-section "Sample Preparation and Pre-Incubation". The micro-organisms are added to the casein soy bean digest broth (SCDB) immediately before or after the product to be examined is added. The micro-organisms are therefore present during the whole resuscitation period of 2 - 5 hours.

I imagine it would be an identical response for the same test in <2021>.

A2: I add the 'bioball'* to the TSBT before the 2-5 hours. It should be inoculated at the same stage that the product is added.
So 10g of product in to TSBT, shake, add 0.1ml 'bioball'*, leave for not more than 2 hours, sub in to EEB.
We would do two separate tests, one inoculated with P.aeruginosa and one with E.coli to cover both BTGN and Ent

*other organism suppliers and methods are available.

VALIDATION OF COLONY COUNTER

I want to know what parameters I have to include in the validation of a automated colony counter which has two modes: colony counter and antibiotic zone reader.

A1: First you must see if your software has data auditing, if so, the software must be validated as category 4 onwards; This process will take into account different tests, according to the requirements of the users, and the measurable parameters, you must remember to adjust the measurement pixel and the type of agar and light intensity, also in the case of halo measurement according to halo from the cylinder This will be supported based on the antibiotic, concentration and strain used.

I have the scan 1200.

microbial limit test query

Microbiological examination test methods for Microbial Enumeration and specified Microorganisms referenced in USP chapter <61>, <62> : 1:10 dilution of the product will be prepared for determination of Total Aerobic Microbial Count, Total Yeast and Mold Count (1 ml + 15-20 ml media in 90 mm plates & incubate) & 1:100 dilution will be prepared from it & incubate for specified microorganism test.

But my API (corticosteroid) Product is water insoluble so I can perform the test as below for more satisfaction. 1:10 dilution of the product & 1:100 dilution will be prepared. 10 ml of (1:100 dilution) + 100 ml media in 150 mm plates & incubate for Total Aerobic Microbial Count, Total Yeast and Mold Count 1:10 dilution incubate for specified microorganism test. Next procedure follow as per <62>. your suggestions?

A1: Because your cream is insoluble in water, have you tried taking 10 grams of product and mix it in 10 grams (1:1 dilution of Polysorbate 20 or 80) and then add this 20.0 gram mixture to 80.0 milliliters of Buffered NaCal Peptone Solution with 4% Tween 20 or 80 and 0.5% Soy Lecithin to achieve a 1:10 dilution that would neutralize the antimicrobial activity of the product? 1.0 and 0.1-milliliter aliquots can be taken from this dilution to have 1:10 and 1:100 dilutions of the test sample in diluent.

USP Bile-Tolerant Gram-negative Bacteria Testing

In reviewing USP Chapter 62, it is stated to use Escherichia coli ATCC 8739 as a test organism for conducting suitability testing for detecting the presence of Bile-Tolerant Gram-negative bacteria in test samples. It should be noted that all members of the Enterobacteriaceae family are bile-tolerant organisms which includes all coliform bacteria, Escherichia coli and Salmonella. It should also be noted that Pseudomonas aeruginosa is also a bile-tolerant organism and is able to grow in Enterobacteria Enrichment Broth Mossel. In addition, Pseudomonas aeruginosa will appear as colorless to gray colonies on Violet Red Bile Glucose Agar. It has to be realized that this test is not 100% selective for just detecting the presence of Enterobacteriaceae in a test sample. I know that Pseudomonas aeruginosa will not give the typical presumptive color reaction on Violet Red Bile Glucose Agar such as members of the Enterobacteriaceae (Pink colonies with red precipitate). However, I'm not a big believer in using just presumptive color reactions on differential/selective growth agars when conducting microbial limits testing of a nonsterile test sample.

Because USP 62 uses Escherichia coli ATCC 8739 as a suitability test organism for detecting the presence of Escherichia coli in a test sample, I wonder why the USP/EP did not recommend a different Bile-tolerant Gram-negative bacterial strain for conducting suitability testing for detecting the presence of Bile-Tolerant Gram-negative organisms such as Cronobacter sakazakii which had been recently implicated in a recent baby powder product recall in the United States or a different coliform organism than Escherichia coli. The only reason that I can think of as to why the USP/EP had selected Escherichia coli as a suitability test organism for Bile-Tolerant Gram-negative bacteria was to either limit the number of suitability test organisms that are present in the chapter or they copied one of the growth promotion organisms that are recommended by media manufacturers. Does anyone include additional bile-tolerant Gram-negative bacterial strains such as other members of the Enterobacteriaceae when conducting suitability testing for detecting the presence of bile-tolerant organisms? Just wondering.

Microbial Test Requirements for Pre and Postbiotics

Are there separate requirements for microbial testing of products that contain prebiotics and postbiotics? Or would this fall under traditional compendia testing for microbial limits?

Qualification of Microbiology Methods for early stage (Tox and Phase 1) product

Can someone tell me where I can find guidance for what the expectations are for qualifying Micro test methods (Bioburden and Endotoxin) for early phase, to Biotechnology derived articles? These are for tox studies and potentially Phase 1.

A1: If you are using a compendial method for microbial enumeration, bacterial endotoxin assays, and sterility testing, the methods described in the official test methods are considered validated and the methods are qualified for a specific product using the method suitability testing as defined in the compendial chapters.

In early phase development, a method employed is selected based on your experience with similar products with the expectation the method will work and may not require a full scale method suitability study that usually requires three independent determination preferably using three lots of product.

This is justified due to the absence of three lot, small lot size that is consumed by the testing, the test material may not resemble the proposed dosage form, and development work will be discontinued due to toxicity, adverse reactions, or lack of efficacy.

The expectation is the manufacturing processes and analytical methods will be fully validated prior to the production of phase 3 clinical trial materials.

I believe this is the industry practice and the regulatory expectation and may be found in industry position papers, FDA guidance documents,ICH standards, and podium presentations.

A2: The main thing is that you evaluate your product at the level of formula and unit process.

Understanding the composition will allow you to know the analytical difficulties and what methodology to use.

In any case, if you are talking about bioburden and I assume that it is a sterile product, establishing the limit based on the terms of the sterilizing filters is a job of collecting process data, there are many regulations that could help you.
At the level of endotoxins, it is important to evaluate interference. Beyond that, the methodology is simple.

A3: Thank you for your response. To clarify we are expected to perform bioburden in process and DS samples for one or two small batches. Due to the small batch size, it is unlikely that we will get anywhere near enough sample to be able to verify or qualify the method. You mentioned in your response that at this stage the method may not require a full scale suitability study. My question is: Would there be expectation of a partial scale suitability study? (I'm not sure how that would look; smaller test sample sizes, or a smaller battery of challenge organisms perhaps). Or just holding off on verification studies until a later phase? I have done searches in the FDA guidance documents and PDA technical reports and haven't found much guidance.

Microbial method suitability <61> and <62>

I have a few questions relating to performing method suitability.
I have had a few push backs from a new customer, we are looking to start testing for, about our expectations with regards to method suitability testing.

1. Where does it state that three independent tests or batches are required to be tested as part of method suitability?
2. I believe that this is generally what is done, and we state this, but i cant see a clear statement of expectation within any pharma chapters or guidance documents.

1. In <1227> it states that 'use of compendial methods requires establishment of suitability of the method demonstrating recovery of the challenge organisms in the presence of the product'. If you are transferring the method from one laboratory to another where does it state that each testing lab must do

this? Do you have to perform suitability at the new lab or can you argue that it has already been done? i cant see anything to state it must be done for each lab.
2. I believe that it should be done, and what we usually do, but im trying to point to what states this.

A!: The USP general test chapters provide the minimum requirement for conducting the tests.

The issues you bring up are related to industry practice and GMP expectations.

A2: In honor of our illustrious creator of the PMFList, Scott Sutton…and forgive me if I don't quote him exactly...."1 is a fluke, 2 is a coincidence, 3 is SCIENCE!" Due to the variability in microbiological methods, 3 is the number the industry has settled on to show the variability in the method is within the limits. We perform 3 consecutive media fill runs, 3 lots for method development and many firms perform testing on 3 lots of products from vendors prior to using them. Remember, statistically, microbiology methods are limited because we can't test the entire product and microbial contamination isn't homogenously distributed throughout a product/process. We try to repeat recovery/inhibition with three lots to show as much statistical significance we can without too much burden on the MFG or the testing lab. A perfect example of this is the sterility test where we know from statistical analysis that if 5% of the batch is contaminated, the test only has a 63% chance of detection because we only take 20 units. Often times firms will test one lot and move forward while testing 2 other lots as they become available. This of course is based on a risk assessment of the product and method.

A3: We do routinely follow what we also believe to be the standard expectations within industry. However the customer is asking for definitive statements that can be quoted. If we cant provide this then they believe that it is not required and can not be enforced.

The biggest one for me is that we still must perform method suitability at our site even though it has already been performed by a different contract testing lab. However this obviously has a cost and lead time to complete so they do not feel it required as it has been done, unless we can prove otherwise to them that it must be repeated by us in our lab with our materials and media etc.

Rather than just saying we believe that this is an expectation and during inspection it will most likely result in an observation/483. However, again if it is not defined then how can it be enforced?

A4: You can perform a risk assessment to demonstrate the receiving lab for the procedure (as shown via method suitability) will be performed exactly as the initial lab that developed the procedure. This is similar to a tech transfer process. I don't know of any written requirement that method suitability has to be repeated, since the actual method has already been validated (i.e., it is a compendial test).

The risk assessment can include your requirements for routine media qualification, etc.

A5: Also the 2008 FDA Guidance Forindustry CGMPs for Phase I Investigative Drugs.

A6: And PDA TR No. 56 (revised) Application of Phase Appropriate Quality Systems to the development of Therapeutic protein Drugs etc 2016 is useful.

Dynamic Airflow Visualization Studies

I have some regulatory questions about dynamic airflow visualization studies. I stipulate they should be done as a valuable process design/risk assessment tool no matter what. I'm more concerned with the regulatory requirements pertaining to phase of development and the type of product.

Are they required prior to phase I production?
Are they required for any open manufacturing in a Grade A environment, even if the final product at that point is not required to be sterile (e.g. low bioburden bulk).

E. coli Presence in 10g but absent on Violet Red Bile Agar

We run analyses on dietary supplements (in this case Spirulina powder) for Enumeration of Coliforms as well as presence/absence of E. coli in 10 grams. Per USP <62> for E. coli and FDA BAM Chapter 4 for Solid Media Method – Coliforms.

We had several lots fail for the presence of E. coli and even had PCR confirmation that the microorganism was E. coli but we resulted in None Detected for the Coliforms. Every lot of Violet Red Bile Agar we make we also perform a growth promotion using E. coli to confirm that the media is suitable for coliform growth. On every analysis we perform we also have a negative control in place that is treated similarly like the sample and those had no growth as well.

Now our vendor the Spirulina is testing their reserve samples using a “Modified USP <62>” from their contract lab as well as their internal testing showing that they use AOAC 991.14. Both methods the vendor is providing data for is showing an absence of E. coli.

Any insight would be appreciated!

A1: It is my understanding that Spirulina powder is obtained from a blue-green algae called Arthrospira platensis. Generally, this blue-green algae is cultivated in un-chlorinated ponds that have a pH around 8 to 10. The blue-algae is harvested, pressed, dried and grounded to a fine powder. It is possible that the source of the Escherichia coli contamination in the powder could be from the usage of un-chlorinated water that is used for cultivation.

Instead of using USP 62, I would think that you would instead be using USP 2022 Microbiological Procedures for Absence of Specified Microorganisms – Nutritional and Dietary Supplements. However, I do not see any harm if you use USP Chapter 62 for detecting Escherichia coli in a nutritional or dietary supplement.

Because microbial contamination of a batch is never homogeneously distributed, I suspect the difference in the microbial results may be due to this reason. I suspect that you are sampling several containers of a batch of Spirulina powder for microbial testing by either testing each sampled container or testing a composite sample from several containers while the supplier may be only sampling one container of the batch that was found to be free of Escherichia coli. It would also be interesting to see how the contract laboratory had modified USP Chapter 62. It is also my understanding that AOAC 991.14 is a Petrifilm method for counting the number of coliform bacteria and Escherichia coli in a sample in which a 1.0-ml suspension of the product is placed onto the Petrifilm for incubation. AOAC 991.14 is not an enrichment method like USP Chapter 62 or USP Chapter 2022 but could be used as a substitute for USP 61 for determining the number of coliform bacteria and Escherichia coli that is present in a sample. Howber, I still recommend suitability testing of AOAC 991.14 if Petrifilm is used for determining a coliform and Escherichia coli count.

Environmental Management

Can folks recommend articles re. EM in pharma context?

A1: PDA TR No. 13 (Revised)

A2: Here are some other resources on EM:
Reducing Risk in Your Environmental Monitoring Program | Pharmaceutical Outsourcing - The Journal of Pharmaceutical & Biopharmaceutical Contract Services (pharmoutsourcing.com)<https://www.pharmoutsourcing.com/Featured-Articles/564781-Reducing-Risk-in-Your-Environmental-Monitoring-Program/>

Reducing Microbiological Risk and Using Quality Tools in the Pharmaceutical Industry (biopharma-asia.com)<https://biopharma-asia.com/webinars/reducing-microbiological-risk-using-quality-tools-in-the-pharmaceutical-industry/>

Best Practices for Environmental Monitoring and Risk Management | Charles River (criver.com)<https://www.criver.com/resources/webinar-pi-ms-best-practices-environmental-monitoring-and-risk-management>

Environmental Monitoring: Identify & Track Resident Microbes with a Contamination Control Program | Charles River (criver.com)<https://www.criver.com/resources/webinar-pi-ms-environmental-monitoring-identify-track-resident-microbes-contamination-control-program>

Welcome to the MicroLearning Microbiology Series | Charles River (criver.com)<https://www.criver.com/resources/welcome-microlearning-microbiology-series>

Medical grade gamma irradiated silicone as a lubricant

I was wondering if any aseptic process experts might have a recommendation here. I'm assisting a firm that has an issue with silicone coated coated stoppers getting "stuck" as they are making their way down the spiral stopper bowl track, causing otherwise unnecessary intervention. An issue that I've seen myself a number of times in my travels. The firm noted that wiping the track with IPA wipes that purportedly contains the same type of silicone (medial grade, gamma-irradiated) in the wipe, seems to lubricate the track enough to greatly remediate the issue. The firm is questioning whether they could introduce this type of silicone as a lubricant, to the stopper bowl (or otherwise?) to the process in order to have a better and more permanent fix in place. Would anyone in the group have a suggestion or insight regarding this?

Hold time prior to terminal sterilization for aseptically filled units

I have a question on the FDA's guidance for determining a hold time after filling but prior to terminal sterilization.
Validation studies are performed on bulk solution up to 48 hours. The product is sterile filtered and aseptically filled.
It is then ALSO terminally sterilized.
Are companies who fill in this way also doing studies to show that after aseptic filling/sterile filtration, the amount of time prior to terminal sterilization needs to be validated?

A1: The hold time for a sterile filtered, aseptically filled pharmaceutical drug product can be three years at room temperature so you may select any hold time you like.

However, the regulatory expectation is your company justifies a hold time based on a stimulation.

Hold time studies for reconstitution and dilution studies for injectable products are based on inoculating the product with 100 cfu/mL of a laboratory culture and determining the time at the storage temperature that the count exceeds 0.5 logs. A ridiculous stringent requirement.

I would conduct a media fill and based on satisfactory results, i.e., a contamination rate less than 1 per 10,000 argue for a week as the holding time. No lab studies.

With the current emphasis on continuous manufacturing the filled vials should be conveyed, and loaded into a water cascade autoclave so there is no hold time.

A2: Yes, the time after filling and before terminal sterilisation is validated and followed. I remember FDA investigators asking for this study and to specify the time in the batch records.

A3: I have some follow up...

We run media fills as required by guidance and incubate for the two-week time period with no failures. This supports the processes and give us the worst case.
We were asked to do a study for up to 168 hrs of hold time (between sterile filling and TS) using a very low-level bioburden (NMT 100 CFU per container (not per mL)). The explanation being that if a vial got contaminated somehow along the way we can demonstrate we would not over-burden the autoclave's capabilities.
In the beginning we were using the 0.5 log increase as our cutoff, but if we only put in 50 CFU, we would then fail if we had 150 CFU (3x give or take) and that was silly since the autoclave is validated at an SAL of 10-6.
What do you think of that?
What do you mean by reconstitution and dilution studies?

A4: How did you validate your autoclave TS process to an SAL of 10-6? Was this using an overkill approach with biological indicators, the bioburden method or combination method?

I ask because if you validated your TS based on the bioburden method, you would have performed bioburden studies to establish the maximum number of bugs in the vials that would accommodate the calculated SAL after the full autoclave cycle. If that is the case, the Agency may be interested to know if a contaminated vial, during the maximum hold period, would exceed the concentration of organisms you relied on during the TS validation studies.

But, if you used the overkill approach, if a contaminated vial promoted an increase in bioburden over time (and before TS), the question would be if the bioburden exceeded the population used in the biological indicators, and whether the heat resistance of the bioburden was greater than the BI spores utilized during your studies.

Discussing these points, If you can, would help to better understand why you are being asked to conduct these studies and whether the studies are warranted.

A5: The result will depend on whether the product supports the growth of microorganisms and is largely irrelevant to the sterility assurance of the product. Contamination of an aseptically filled vial is a very low probability event. All vegetative microorganisms and almost all spore-forming bacteria are very readily killed by an autoclave cycle. In seconds not minutes.

Without being too insulting they are not knowledgeable.

Using a validated aseptic filling process followed by a terminal sterilization will result in an incredibly safe injectable product and a reasonable time limit should be selected based on manufacturing contingencies.

A6: Wait a moment please. If the autoclave is qualified for 10x6 SAL doesn't that already answer the question of "if a vial got contaminated somehow along the way we can demonstrate we would not over-burden the autoclave's capabilities"? Less than 100 colonies is less than a million, last time I counted. This request seems completely unreasonable unless they don't accept the validation studies that were done. I would respectfully decline to do the study.

A7: The answer to "who asked" is gray. There is an FDA QbR for Sterility Assurance of Terminally Sterilized Products.
In it there is a "mock" question:
Are there validation studies that support holding periods of the bulk solution after compounding or of the finished drug product after filling but prior to TS?
Then there is the actual FDA presented question:
Q: Are bulk hold studies necessary for products that are terminally sterilized, and if so, what information should be included in this section?
A: Depending on the drug product attributes (such as promoting growth of microorganisms), the length of holding, the conditions under which the bulk solution or filled product is held, and any additional manufacturing steps used to reduce bioburden prior to holding (such as filtration), studies may be necessary to support any holding periods of the bulk solution or of the filled drug product that might

contribute to excessive growth of microorganisms. Although the terminal sterilization process may kill microorganisms, the sterilization process cannot remove endotoxin and other released toxins and metabolites that could result from microbial growth during extended hold periods.
Validation study information should include:
- Date(s) of performance and study report numbers •
- Holding conditions of time, temperature, storage vessel •
- Indication if the storage vessel is sterilized prior to use
- Description of any bioburden reducing steps used prior to storage (such as pre-filtration)
- Description of sampling plan
- Description of how the samples were assessed for growth
- Acceptance criteria for the validation study
- For holding periods not validated, include a scientific justification for not performing these studies.

I personally did not interpret this to equate to the study that we are performing, I feel it pertains to bulk hold time or studies on products that rely solely on TS for confidence in sterility. But at some point, someone did and agreed to begin performing these studies. Once you do a study and submit to FDA one time, you're basically signing up to do them forever, and here we are. I have argued the point on deaf ears, so we truck on. This is why I brought it up here - to see what other companies are doing.

A8: These are the GMP requirements for time limitations

21 CFR 211.111 *Time Limitations on Production* states: When appropriate, time limits for the completion of each phase of production shall be established to assure the quality of the drug product. Deviation from established time limits may be acceptable if such deviation does not compromise the quality of the drug product. Such derivation shall be justified and documented.”

2004 FDA Guidance for Industry - *Sterile Drug Products Produced by Aseptic Processing — Current Good Manufacturing Practice* states: “When appropriate, time limits must be established for each phase of aseptic processing (§ 211.111). Time limits should include, for example, the period between the start of bulk product compounding and its sterilization, filtration processes, product exposure while on the processing line, and storage of sterilized equipment, containers and closures. The time limits established for the various production phases should be supported by data. Bioburden and endotoxin load should be assessed when establishing time limits for stages such as the formulation processing stage. “

“The total time for product filtration should be limited to an established maximum to prevent microorganisms from penetrating the filter. Such a time limit should also prevent a significant increase in upstream bioburden and endotoxin load. Because they can provide a substrate for microbial attachment, maximum use times for those filters used upstream for solution clarification or particle removal should also be established and justified.”

The key wording is when appropriate that requires the exercise of judgement.

A9: Thanks for the FDA guidance reference.

Your bioburden control prior to terminal sterilization is sterile filtration and aseptic filling so with the completion of a media fill validation you have conducted the hold time study as required by the FDA. This validation study will justify a shelf life or an injectable product at room temperature for 3 years. The sterility assurance level is high. Companies routinely fill >30,000 vials of medium, incubate, and have no turdidi vials. A hold time of a week would be fully justified.

Preservatives in non-sterile aqueous product

We have a product under development where BKC is used however we are looking to potentially change this to an alternative for assessment.
I am looking for information on the most commonly used preservative within aqueous nasal spray products and what peoples experiences with them are so i can review them.

Also, does anyone have any reference sources or book names etc relating to this topic?

A1: There are several preservatives to use instead of BAK and BZT. I am assuming that BKC is BAK, benzalkonium chloride. Formulating nasal sprays is easy. You need to also watch the other ingredients in the formulation and pH. I prefer using the preservatives found in ophthalmics, for instance. Highly effective at low concentrations. Keep in mind that you must also seek formulation development around the re-use of the applicator and potential contamination of the contents during re-use. There are additives to make the nasal sprays more effective.

A2: In general, I have found that the most common preservative system that is used in a nasal spray product formulation is Benzalkonium chloride with or without Disodium EDTA. However, I have also seen the use of Potassium sorbate and Sodium benzoate, Benzalkonium chloride, Benzyl alcohol and Disodium EDTA, and Methylparaben, Propylparaben with or without Disodium EDTA as the preservative system in different types of currently marketed nasal spray product formulations.

Besides the published reference books by J. Kabara and David Steinberg on preservatives, I have never seen a reference book indicating what type of a preservative system is commonly used in different types of product formulations. If such a reference book was ever available, I would think that it could be a great reference book. However, you have to realize that there is alot of product chemistry based upon what ingredients are present in a formulation that is involved in selecting a preservative system that will work in a product formulation besides just conducting microbial challenge testing on a formulation.

As an aid in your research, I would suggest that you can look at the ingredient product labels for different types of nasal products in a drug store to see what type of preservative systems that people are using in their nasal spray products to protect against microbial contamination during usage. It is a great way to gather intelligence as to what your competitors are using as the preservative system in their nasal spray product formulations in the absence of a reference book that lists the different types of preservatives in product formulations.

A3: Different preservative but interesting phenomenon re. cepacia from water system.
Factors Affecting Survival of Pseudomonas cepacia in Decongestant Nasal Sprays Containing Thimerosal as Preservative

https://www.sciencedirect.com/science/article/abs/pii/S0022354915443849

Glyceryl caprylate as a Preservative

I have a question about the usage of Glyceryl caprylate as a preservative in non-sterile product formulations. Glyceryl caprylate is a natural preservative obtained from coconuts or palms and has excellent antimicrobial activity against bacteria and yeast. It is my understanding that the recommended usage concentration for Glyceryl caprylate in product formulations as a preservative is between 0.3 to 1.0%. However, I have never used this ingredient at a concentration greater than 0.5% in a product formulation. However, I have been hearing that concentrations of this ingredient at between 0.6 to 1.0% in a leave-on product formulation could be quite irritating to the skin and leave it flushed red. I never had an irritation/sensitivity issue when I had used this ingredient in a product formulation at a concentration below 0.5%. Has anyone experienced the same issue of skin irritation and redness when this ingredient is used as a preservative at a concentration greater than 0.5% in a product formulation?

A1: Are you possibly hydrolyzing something in the formula during a pH adjustment? Are you performing a pH adjustment at an elevated temperature? I don't think that this ester would cause much irritation. It is a slight surfactant as well. Could something else in the formulation be involved?

Papers show the ability to use this ester up to 15% without issue. I will suggest finding a reaction within the formula that may be causing the reaction. Further, is the quality of the material of good quality. Could you have an allergen (protein) in one of the materials? Could changing sources help?

Personally, we have not formulated or tested this ester in a formulation greater than 0.5% to date. Interesting response that you are obtaining.

A2: Thank you for your response. In general, I have used Glyceryl caprylate in formulations at a pH of 4 to 7 with no problems. It is my understanding that this ingredient will hydrolyze in a formulation after a pH of 7.0. I have never added this ingredient into a formulation above 60C. According to the Cosmetic Ingredient Review, they stated glyceryl monoesters such as Glyceryl caprylate are safe as a cosmetic ingredient and are not irritating at the concentrations used in cosmetics.

I can see this ingredient as an irritant to eyes, but not to the skin. I have read that a 15% concentration of Glyceryl caprylate did not cause skin irritation or sensitization in RIPT studies. In conclusion, I'm a little confused as to why someone told me that concentrations of this ingredient greater than 0.5% will cause skin irritation and sensitization issues.

As to what you had suspected, I believe that there is another ingredient in the formulation that is causing the problem. When it comes to skin irritation or sensitization, the first thing that sensory and toxicology people blame the problem on is the preservative in the formulation.

Reclassification of ISO Class 7 Cleanroom to ISO Class 8

There is a proposal to reclassify our ISO Class 7 cleanrooms to ISO Class 8.

Apart from the following, what else needs to be taken into consideration?

1. Air balancing, recertification and revalidation of the cleanrooms

2. Change to less stringent gowning requirements

3. Assessing impact on irradiation sterilized medical device products and redo sterilization validations on affected product families

4. Regulatory approvals

Any suggestions are welcome.

A1: I would consider looking into the following:

1. Does your facility have pass thrus? Although not a rated space, still good to assess how this change will affect movement of materials
2. Possibly decommission equipment that can no longer be used in ISO 8 setting
3. Cleaning adjustments such as the agents used and frequency
4. Environmental monitoring (air and surface sampling) - part of the recertification

Just some ideas although I have never done this process of going from ISO 7 to ISO 8.

USP 61 Surface Spread reporting results question

Looking for clarification on reporting results using the surface spread method in usp 61. We have asked the question below to the USP liaison at the time of the email and I have included their response.

Email Question to USP:

We are having difficulties understanding the interpretation of USP <61> section "Surface Spread Method" under the Recovery of Microorganisms in the Presence of Product. The section states to: Spread a measured volume of not less than 0.1mL of the sample, prepared as directed under Preparation of the Sample, Inoculation and Dilution, and Neutralization/Removal of Antimicrobial Activity over the surface of the medium. Incubate and count as directed for Pour-Plate Method. We understand this as follows: 10g sample into 90mL PBS to make a 1:10 dilution; plate 0.1mL of 1:10 dilution, results are reported (if no growth) as <10CFU/g. If any growth is found it would be the # of colonies multiplied by 10 (the initial dilution factor). Are we correct in calculating this way? Any insight you can give is greatly appreciated!

USP Response from the Principal Scientific Liaison-General Chapters:
Yes-your interpretation is correct!

A1: There is a slight error in your example calculation. The plated aliquot volume needs to be taken into account, not just the dilution.

In your example, 10g into 90mL PBS is 1:10. 1mL of that 1:10 preparation will contain 0.1g of your sample and a no growth result would be reported as <10 cfu/g. However, by plating 0.1mL of that 1:10 preparation only 0.01g of your sample is being represented on the plate therefore a no growth result would be reported as <100 cfu/g.

Figure out the amount of sample being represented on the plate and take the reciprocal of that value to determine the test limit if no growth is observed.

A2: Robert is correct and this has been a difficult concept for people to understand when training them on this method. Using the theory of "how much sample goes onto the plate/incubator" is a great way to ensure you don't miscalculate.
We have incorporated the explanation in our procedures as well as examples of how to document the results so that there is no confusion in results reporting. Any time the procedure requires spread or pour (pour at 1.0mL and spread at 0.1mL) we apply an automatic 1:10 in the calculation and that has made things a little bit easier. Then, of course, you would perform your usual back-calculation for dilutions, etc.

BCC in Water - Warning Letter

A recent warning letter to a facility in St. Louis noted the following observation from FDA:

"Additionally, you have detected Burkholderia cepacia (B. cepacia) in your finished drug products on numerous occasions. Since B. cepacia is a waterborne organism, this recurring product contamination further indicates the impact of insufficient monitoring and control of your water system. You did not have appropriate limits to identify an adverse pattern of B. cepacia in your water system, nor did you routinely test your water system for the presence of B. cepacia utilizing validated methods."

I'm very interested in the second part of the last sentence that mentions "... nor did you routinely test your water system for the presence of B. cepacia utilizing validated methods."

I have a few questions regarding this (which has been noted in other FDA observations in 483s and Warning Letters):

1. I wonder if this is a backdoor endorsement/requirement by FDA to require water systems to be tested using USP <60>, Burkholderia cepacia complex. I am unaware of other "validated" methods for Bcc as there is nothing published in publications such as Standard Methods or AOAC or other references. Please note that USP removed the term "water" from the draft versions of USP <60>. The chapter now states 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." I agree that water is a substance in aqueous preparations so I can understand the Bcc test requests but are people actually using USP <60> to test their purified water?.

2. Have people tried other tests methods such as membrane filtration of the water directly onto BCSA or other media? If so, how is that methodology being "validated"?

A1: Many of my non-sterile aqueous product clients who have had issues with BCC in finished product or unit operations routinely include USP 60 when testing water samples. In a number of cases, FDA has asked the same questions. I plan on addressing some of these issues during the PDA microbiology conference in October.

A2: In many of the warning letters the company made a nonsterile liquid product, justifying the concerns about BCC.

I haven't seen a similar comment for companies only making sterile products.

It was easy to validate equivalent growth of BCC on R2A.

A3: Interesting observations.

USP <60> was written specially for screen aqueous, non-sterile drug products and as an official compendial test method meets the requirement of being a validated method. Members of the B. cepacia complex can be isolated on R2A agar so you do not need to look too far for a method. As a APHA/AWWA standard method it is considered validated and will pass a growth-promotion test using B. cepacia.

I would not recommend monitoring purified water with a selective medium.

Pharmaceutical water systems were never expected to deliver water with an absence of Gram-negative, oxidase-positive bacteria.

Manufacturers will need to manage their water systems better, formulate their products to be robust, and control their manufacturing processes to exclude objectionable microorganisms including Bcc.

A4: I've asked this on 3 separate occasions with FDA and USP officials. The "official" word is that pharmaceutical companies, in general, don't need to test their water for Bcc. Then, about 4 years ago, a large pharma company recalled their oral solid dose due to finding Bcc in their production waters (or at least in the process). While USP 60 isn't applicable to OSD, that firm chose to take the very conservative route. Since then, I've noticed other Bcc contaminations and 483's in areas that didn't seem applicable, but Bcc was there nonetheless. Also, here was the final USP 60 verbiage:

Prepare a sample using a 1-in-10 dilution of NLT 1 g of the product to be examined. Use 10 mL or the quantity corresponding to 1 g or 1 mL to inoculate a suitable amount (determined as described in Suitability of the Test Method) of Soybean–Casein Digest Broth or an appropriate dilution of Soybean–Casein Digest Broth as determined during method suitability (for example, a 1:10 dilution may be required when conducting optional testing of pharmaceutical waters). Then mix and incubate at 30°–35° for 48–72 h.

I specifically asked about diluting the TSB for pharmaceutical waters and that was indeed the interpretation. So there is no direct to BCSA method and I don’t think it would work without an enrichment step…but that’s my personal opinion. I often talk about your original APR article and I’ll reference it for people here: https://www.americanpharmaceuticalreview.com/Featured-Articles/160451-Recovery-of-Stressed-Acclimated-Burkholderia-cepacia-Complex-Organisms/

I do believe many firms who MFG non-sterile liquids test their waters for Bcc and if they do find it in the water, but don't investigate the MFG process, that has generated 483's.

A5: FDA and CDC spoke on routine BCC testing of products made with Purified Water systems in 2018 at AFDO. I know that the question was asked if routine testing of BCC was a requirement. The response was, "it depends. How are you verifying routinely the source materials for potential contamination? If it is a good idea and it makes sense, the GMPs are about common sense." The words may not be exact, but they are close. Following this presentation, Marla Phillips presented on the plans developed at Xavier on supply chain KPIs. I followed with a presentation on supply chain issues for Compounding Pharmacies. I did bring up BCC issues in my presentation. I did receive a few smiles from senior FDA management.

FDA did say on Monday of this week, they are coming in full force on September 1. I am guessing that we should have fun. The consultants and contract labs should be smiling.

I know several FDA officials read these comments. I hope that someone will squawk.

A6: I agree with your comment that it seems that these FDA Warning Letters are being used as a backdoor endorsement/requirement that purified water systems be tested for the presence of Burkholderia cepacia complex by using USP Chapter 60. I do believe that purified water systems should be tested for the presence of Burkholderia cepacia complex because they are the major root cause of Burkholderia cepacia contamination of aqueous finished products.

You are also correct that there are no published validated methods for the isolation of Burkholderia cepacia complex from water by either AOAC or APHA which is responsible for Standards Methods. For conducting microbial analysis of purified water samples, I would think that most people are using a method that is present in Standard Methods. The closest publication that I have ever seen validating the isolation of Burkholderia cepacia complex from water is the paper that you had written with Scot Sutton. It would be interesting to hear how people are validating their Burkholderia cepacia complex test methods of purified water samples.

A7: This is definitely an interesting issue, given the clinical significance of Bcc members and their association with insufficiently maintained or insufficiently monitored water systems. I'm curious to hear insight from those with more knowledge about the development of USP <60> on why 'water' was removed from draft versions, with a focus seemingly more on end product testing.

Interestingly, the chapter does mention 'optional testing of pharmaceutical waters'.

TESTING OF PRODUCTS
Sample Preparation and Pre-Incubation
Prepare a sample using a 1-in-10 dilution of NLT 1 g of the product to be examined. Use 10 mL or the quantity corresponding to 1 g or 1 mL to inoculate a suitable amount (determined as described in Suitability of the Test Method) of Soybean–Casein Digest Broth or an appropriate dilution of Soybean–Casein Digest Broth as determined during method suitability (for example, a 1:10 dilution may be required when conducting optional testing of pharmaceutical waters). Then mix and incubate at 30°–35° for 48–72 h.

In my previous experience at a nonsterile CDMO, FDA auditors were keenly interested in any Gram negative rods (esp. Bcc) in our water testing data. For good reason, to be honest, since the facility had some past 'events' of contaminants in their previous (per production room) water systems. The site agreed to test for Bcc in water and products (this was about 5 years ago or so), so before USP <60> was in effect. Our validated method for Bcc testing was very similar to what was in the chapter, so compliance with the chapter was pretty smooth. We eventually installed and validated a water system to serve the entire facility, and continued testing for Bcc. We did get hits early in validation, and that allowed us time to tweak

system maintenance and sampling procedures to a point where we operated without any Bcc hits in the distribution lines and POU drops during and after our last validation phase.

I'm not sure why USP <60> isn't pushed more for water systems, given the opportunity for early detection of problems. Detection of Bcc during end product testing in the QC Micro lab is too late and results in more lost product and risk to the business. In addition, without early detection in a water system and the uneven distribution of microbial contamination in a batch, there is also the risk of releasing products that have undetected Bcc contamination. On the other hand (and I think this was discussed fairly recently on the PMFList), companies could also adopt an 'ID all' approach when it comes to finding growth on nonselective agar (PCA/SMA, R2A) to detect Bcc in the system on the purification or distribution sides.

Regarding point 2, the short answer is I don't know (I know, real helpful). My thought is that given the likelihood of water system organisms being in a stressed state and the inhospitable nature of BCSA, I'm not sure how effective that would be without an enrichment step. However, I would also be interested to know if anyone's been able to validate that method, though.

That's my perspective on all this, and I'm excited to hear other's thoughts on this issue.

A8: The FDA expectation is to test (purified) water systems for BCC to show the system is under control ('aqueous' is a euphemism for water). Membrane filtration is possible as long as you prove recovery, similar to any other organism (running positive and negative controls).

A9: If you read the email thread you will see I have already answered your questions.

Another possibility is to use replicate plating from a spread or membrane filtration plate onto BCSA to determine if any of the colonies are members of the Bcc.

A10: I understand all that, my question is how is really what the FDA considers a "validated" method and how are people going about that for purified water systems. I know that Standard Methods and USP <60> are considered validated. That's why I'm thinking there seems to be an indirect push to use <60> for water systems. I'd love to see more clarity or even studies on the ability of any Bcc method to recover the bugs from water systems.

A11: I read the FDA 483 you provided slightly differently.

I see the focus being less on the "validated method" and more on the fact that the final drug product was routinely being contaminated with BCC and that the manufacturer had not been looking into the water as a potential source of contamination for this water-borne organism. If a non-sterile aqueous drug product release test pulls out a BCC species, then that root cause investigation should include an evaluation of the water system. If end product testing but not water samples have BCC recoveries then I should be evaluating whether my water sampling procedure is robust enough to detect BCC organisms. I can only assume that the multiple investigations associated with previous BCC in final product were not able to identify a root cause. Thus, the reference to inadequate water testing in the observation. Without knowing more details this is my assumption and interpretation.

So to address your comment #1. No I don't think this should be considered a blanket endorsement of BCC testing for all pharmaceutical water but that it should be done as needed to demonstrate the water is of sufficient quality for it's intended use.

For #2, while at FDA I saw a number of different test methods that were demonstrated to show adequate recovery of BCC organisms on several common nutrient media. As long as the firm has data to demonstrate they can detect BCC should it be present, that should be sufficient for a water system.

A12: I have personal experience with a number of clients who have been using USP 60 for water system monitoring, following BCC contamination events in manufacturing and finished product. Some have followed USP 60 method suitability with their water samples. I know, it's water. I also know that FDA labs have also used USP 60 to test water samples it has collected during inspections.

A13: As in any other compendial method, USP 60 needs to pass suitability testing for the application it's being used. The method is clear about the organisms to use (Burkholderia cepacia, Burkholderia cenocepacia and Burkholderia multivorans), method suitability, incubation, recovery, etc. For suitability of the test method the product is water which should be treated as any other liquid product. Unless I'm missing something, it seems pretty clear to me. Thanks.

A14: I certainly agree with Jessica's position. A failure to exclude Bcc from a aqueous, non-sterile drug product requires an investigation that would include a review of the PW monitoring.

The observations do not request that USP <60> be used for routine water monitoring.

A15: So there seems to be an expectation that the FDA is expecting the use of some sort of (selective) testing of purified water for Bcc and it seems that USP <60> looks to be the main candidate that they are happy with.

Question for everyone - are you guys performing suitability testing using the 3 Bcc bugs as specified in <60> or are you "starving" the Bcc microorganisms as the JP does with Methylobacterium extorquens (ATCC BAA-2500) and Pseudomonas fluorescens (ATCC 17386): “Prior to the media growth promotion test, inoculate these strains into sterile purified water and incubate at 20-25°C for 3 days”? Would that "starvation/stressing" process even make sense? I know in our published study in 2014 with Scott Sutton, we performed recovery studies using the 3 Bcc microorganisms inoculated into 2-8C sterile purified water mimicking the "starvation/stressing" process and showed recoveries over 6 weeks using various media.

I'm also wondering if the USP committees (or any FDA labs) are looking into new Bcc testing methodologies for purified water systems?

Interesting times - especially if you are making an aqueous product and ignoring Bcc microorganisms in your water!

A16: Thanks for your insight (and FDA's experience) on Bcc! I get that the warning letter from FDA hammered the organization for ignoring Bcc issues pretty much everywhere and that it seems they didn't find a root cause for the sources of their finished product contamination. I wonder what methodology they were using for their water testing (I have seen some pretty egregious water testing methodologies in my time!) and whether that method was capable of recovering Bcc at all?

I wonder if the USP <1231>, Water for Pharmaceutical Purposes Section 8.5 Table 3 showing 2-3 day incubation of Purified Water micro testing, may need to be revised or eliminated and a more forceful recommendation of media and incubation qualification studies added. There are plenty of comments about this in section 8.5, however. I especially like these two:

" Every water system has a unique microbiome. It is the user’s responsibility to perform method validation studies to demonstrate the suitability of the chosen test media and incubation conditions for bioburden recovery. In general, users should select the method that recovers the highest planktonic microbial counts in the shortest time, thus allowing for timely investigations and remediation. Such studies are usually performed before or during system validation."

"Cultural approaches are further defined by the type of medium used in combination with the incubation temperature and duration. This combination should be selected according to the monitoring needs of a specific water system and its ability to recover the microorganisms of interest; that is, those that could have a detrimental effect on the products manufactured or process uses, as well as those that reflect the microbial control status of the system."

For our new clients, we recommend a media and incubation qualification study be performed and while many do, a large percentage opt for the 2-3 day SMA 1mL testing at 30-35C as noted in USP <1231>.

Who knew it would be so much fun to be a non-sterile pharmaceutical microbiologist lol!

A17: Rick, my clients have performed method suitability as required in USP 60.

A18: As a microbiologist I am not a big fan of <1231> especially statements on the uniqueness of each water system and the need to qualify the microbial enumeration selected when APHA/AWWA methods are available. As there are not added substances in PW method suitability testing should not be a requirement.

I would use R2A agar incubated at 25 degree C for 5 days and identify representative colonies from the membrane. Using selective media is terrible idea.

A19: I think the method USP 60 describes is adequate and certainly the USP 1231 method would isolate many (certainly not all) organisms from water. Where we even get into MORE gray area is what is "objectionable"? Bcc is one complex of species of known opportunistic pathogens. I think it became obvious more than 10 years ago that this was a very prevalent species in non-sterile, liquid products and the FDA/USP knew we'd have to start monitoring for it, but that doesn't exclude Acinetobacter, Pseudomonas, etc...other species that are also opportunistic pathogens. So USP 60 at least gives us the chance to detect known Bcc opportunistic pathogens that general methods don't (unless you ID all isolates) and other methods can't isolate all the opportunistic pathogens that may exist in pharmaceutical waters, in particular, non-sterile, aqueous products. Where does it end?!?!?

As you said, who knew it would be so fun to be a non-sterile QC Microbiologist!

A20: I am trying to test the purified water with the BCA planning to filter an amount equal to 100 ml on the specific medium and to check if there is growth. I am carrying out suitability tests using TSA - R2A and BCA in parallel and evaluating the numerical recovery of 3 different strains of B. cepacia with excellent results. My intention is to monitor the implant in this way both as a total count on R2A as per routine filtering 10 ml, and as a verification in CA of P. aeruginosa on 100 ml with the filtration method (without pre-enrichment in TSB) and I would also like to rotate the control of B. cepacia by periodically replacing the CA with the BCA. What do you think?

A21: I have been following this discussion. I find the most recent comment by [name redacted] concerning the conductance of suitability testing on purified water samples to be very interesting. In general, it is my understanding that suitability testing is conducted on a sample to demonstrate the neutralization of antimicrobial cidal or inhibition activity of ingredients and/or preservatives that are present in a sample to demonstrate the recovery of organisms from that test sample. [name redacted] is correct that purified water does not have anything added to the water that are antimicrobial unless you are considering the addition of ozone. However, the presence of ozone in a purified water sample should have been destroyed by ultraviolet light before the sample was even collected to prevent the inhibition of the recovery of organisms.

If companies are performing suitability testing on purified water samples by using either USP Chapter 60 or a pour plate or membrane filtration from APHA (e.g., Standard Methods for the Examination of Water and Wastewater), what is the point? Could someone please explain as to why they are conducting this suitability testing in the first place? It just does not make any sense to me unless you are checking off a box for an inspection.

If a regulatory agency had asked, are you able to detect the presence of Burkholderia cepacia complex from purified water samples? It would be an interesting question. For most microbial isolates that are recovered from purified water samples, they are nutrient stressed due to low nutrient levels that are normally present in purified water systems. To demonstrate that you can isolate Burkholderia species in purified water samples, I would suspect that most people would use the 3 Burkholderia strains that are present in USP Chapter 60 that are 18 to 24 hours in age at an inoculation level for each at less than 100 CFU. If a lyophilized culture of the 3 strains that had been rehydrated at less than 100 CFU/0.1-ml, I would also think that these lyophilized strains are also not enough nutrient stressed since they are prepared from 18 to 24-hour broth cultures before they are lyophilized. Would either of these culture preparation methods be adequate to demonstrate that you are able to isolate stressed Burkholderia from a

purified water system when the test strains were not nutrient stressed? From my perspective, I do not think so. I would think that it would be best to use the stress method that Rick and Scot had used by storing the culture at 2 to 8C for a week before use to demonstrate that you are able to isolate nutrient stressed Burkholderia from a purified water system by using either USP Chapter 60 or an APHA test method.

A22: Whilst reading the BCC and 1231 posts in PMF, I was wondering about the thoughts from an industry colleague (and occasional) provocateur, Dr. T.C. Soli, and what he would say. So I emailed him and he advised that he was somehow dropped from PMF list and not aware of the conversation. But he did give me a technical reply. So I make this reply on TCs behalf. His email is enclosed. I would add that some of these points have been previously noted, but TC hasn't seen some of them. TC authorized this reply.

"Regarding R2A, people need to realize that the APHA methods are intended to be used for Drinking Water. And R2A is superior to all other media recommended by APHA as candidates for testing DRINKING WATER! But the "mechanism of action" of this medium is probably NOT its "low nutrient" level as most folks and conventional wisdom assume. R2A has a huge variety of nutrient types in it - something for every organism that might be carbon-source-picky living in organically-impure drinking water. My unproven theory (and that is all it is) is that the organisms in high purity water systems cannot be picky or they would not survive on whatever random nutrient (TOC source) happens to be present from time to time. They have to be "omnivores" able to utilize a very wide variety of nutrients in order to persist in the distribution portion of a pharmaceutical water system. That is a relatively short list of organisms compared to drinking water.

How did I come to this theoretical mechanism? My interpretation of observed phenomena. When you test a water sample using multiple media, such as R2A, PCA, TSA, whatever medium, you get the same microbial count and species most of the time, though you have to incubate R2A much longer since it has a much lower nutrient level than the others and colonies just grow slower. It doesn't seem to matter what the level of the nutrient or the types/variety of nutrients are in the media - going against the conventional wisdom. They all seem to work equally well. Yes, I have seen a couple of water systems (yes, only 2) where R2A is better than others, but most of the time, that system testing included samples from the pretreatment part of the system (which is not routinely done for pharma systems) where the water is much less pure and more like drinking water (where R2A works best) than USP Purified Water. The statement in <1231> where different water systems could have different bacterial populations requiring optimal recovery media for those populations is sort of a veiled way of recommending trying several media to see which works best for recovering YOUR water system microbiome. This is where folks will see that R2A does not shine as the best.

Growth-promotion challenging the media with “stressed” but lab grown organisms is probably a waste of time and does nothing to prove R2A is best. Those stressed organisms could not possibly mimic your real microbiome which originated from water system biofilms. The co-testing of the water system with several media to see which gives the highest count in the shortest time is probably the most revealing approach. Frankly, growth promotion challenges for the media are largely just to make sure the media have been properly prepared, the nutrients are suitable, and not over-cooked or degraded during autoclaving!

So why am I the heretic and "against" R2A? Any user who has used both "high nutrient agar" and R2A to test water knows:

(1) R2A requires longer incubation to see countable colonies. That can be a problem for process control with such delayed data availability.
(2) At 5 day incubation, the typical period for R2A because EP specifies this as the minimum incubation time at 30-35C, some colonies can be pinpoint sized (just specs that could be agar surface imperfections) and easily overlooked, so under counted. This is very common if the media are incubated at 30C (or cooler) instead of nearer 35C but all still within the EP-recommended 30-35C range. If incubating at 25C (which may indeed be more appropriate for water systems that continually operate at temperatures in the 20’s, 5 days is not nearly long enough incubation! 7-10 days or even longer would probably be better to

make sure all colonies that could grow are of a visually detectable and countable size – but that is a very long delay if concerned about process control using these data.
(3) Very often, when the colonies must be sub-cultured to other media for identification, they don't grow (those colonies are referred to as "dysgonic"), so can't be identified - a phenomenon that usually does not occur as frequently with colonies sub-cultured from "high nutrient" media which usually has been incubated for fewer days and recover the same organisms as R2A.

Regarding the USP Chapter <60> test for BCC. Yes, it should be used for testing water systems, though it may not be ideal because of the initial enrichment step in high nutrient broth (TSB, which is very rich compared to the solid culture media listed above because of its sugar content). If the BCC cells present in the water are very low in number, some may not survive the transition to that enrichment step, leading to a potential for false negatives from that test. But regardless of this potential deficiency, that method is still the best around and I commend USP's Microbiology EC for getting it into USP . If you have products that use your water system that are at risk from BCC contamination because of the product properties, how it is used, or immune-compromised product users, then you had better be testing for BCC in your water system and make sure it is absent with good microbial control measures."

A23: This is excellent and T.C.'s observations match much of what we have seen with our many optimal media recovery studies of purified water systems for various pharma organizations around the country. R2A is the best recovery medium in about 1/3 of the systems we perform these studies on. As T.C. mentioned, we are looking for the best recovery in the shortest amount of time, and SMA (or PCA) seems to provide that for the remaining two-thirds of those water systems.
I also agree that "stressed" lab cultures are kind of pointless and do nothing to mimic the recovery of actual microorganisms found in water.

T.C.'s (and [name redacted]) comments about using USP <60> for recovery of Bcc in water are of interest as well. I do think that some studies should be run to determine whether <60> can easily recover Bcc organisms as well as the heterotrophic plate count media that are being used, whether it's R2A or SMA. I know there was some interest in low-nutrient media such as 1/3 strength TSB in FDA labs a few years ago (see "Evaluation of liquid and solid culture media for the recovery and enrichment of Burkholderia cenocepacia from distilled water" https://academic.oup.com/jimb/article/41/7/1109/5995226) that might recover stressed Bcc microorganisms. I wonder if anything went further with those studies? I know Dave Hussong was involved in these.

A24: Most of this is a check the box exercise. In the past I had been asked for a validation. We are actually completing one now, it would be nice to have some language in 1231 that bioburden method confirmation is not necessary.

A25: A nice discussion has opened on the use of R2A on which I agree on the various perplexities described in some emails, in particular on the less rapid growth of colonies compared to TSA for example. But here in Europe the soil described in EP for the control of purified water is R2A, which therefore remains the preferential medium according to EP. But my question was another: to be able to numerically highlight the quantity of B cepacia present in the water and to use BCA in parallel as a selective BCA counting medium, avoiding pre-enrichment in TSB. For years I have been using CA as a counting medium for P aeruginosa in order to highlight any colonies that can be selected in users, for example little used. Filter instead of 10 ml, 100 ml of water to make the method more sensitive. I was wondering if it might be useful to apply the same procedure, that is to filter 100 ml of purified water and put the membrane on BCA to quickly check for the presence of B cepacia with a selective medium. I proposed to make the comparison for a possible validation of the method with R2A because it is the medium I use for the count of purified water, but I could also add TSA in parallel and verify the numerical recovery of some strains of B cepacia in the selective BCA medium . This is for a use of the land as a selective direct count of BCC CFU in the water.

A26: Great comments from T.C. Soli. Thanks for the shout out for <60> that was developed for non-sterile product testing not water monitoring.

For a water monitoring program we must decide what is most important - highest count, greatest diversity, time to result, ease of subculture of isolates, or detection of objectionable bacteria.

Ph. Eur. picked time to result by using R2A agar incubated at 30-35 degree C.

A27: As [name redacted] mentioned previously in the thread, direct enumeration of isolates on selective media, is not recommended.
Selective agents by nature generally exhibit selective toxicity.
"Relative sensitivity" of the bioburden to selective or differential agents , even members of those genera & species for which the media is selective, exists as a spectrum, and direct testing will yield variable results, usually underestimating the actual presence, of the target or target group.
Add to this the impact of various stressors (heat, pressure, Aw, chemicals etc) that might pertain to the source material, recovery would more than likely be less than optimum and results hardly reproducible.
For that reason traditional recovery methods of specific flora usually involve Pre- Enrichment and / or Enrichment of the test sample and are effectively presence absence or semi-quantitative tests depending on how they are employed.
I would recommend replicate plating or subculturing a representative number of isolates from general purpose growth media onto selective media for a quantitative or semi -quantitative estimate.

All
Following the thread, I think it's important to highlight that suitability testing should by nature be a relatively straightforward procedure as it should relate to a well-established pharmacopeial method.
We assume that such methods are well established and validated only requiring verification / suitability?
That we are discussing the "whys and wherefores" of suitability testing for BCC in water is revealing and my feeling is that in mentioning BCC Water testing in USP >60> the USP is too previous and instead it should be referred to in USP <1231> until such time that there is a broad consensus or agreement on the effectiveness of the standard methodology.

Are we putting the cart before the horse?
The requirement to test a material or an article should be based on QbD and QRM principles which should include Objectionability Evaluation and Upstream Microbiological Process Profiling (rarely even mentioned it seems to me).
The Microbiological Profile of a given process together with a suitable Risk Evaluation that includes Objectionability (Pathogenicity, Spoilage, Efficacy, Stability issues) should inform the Contamination Control Strategy rather than the other way around.
The use of experimental or complicated comparative testing should come into play at the upstream, R&D, Product Development stage i.e.. Process Profiling and allow for effective recovery for the given process type.

Do water systems need to be tested for BCC, yes if the process / product is at risk on the basis of "Objectionability" or "Pathogenicity", taking into account all end users, dispensers, compounders, patients etc.

Bottom Line , Manufacturers need to know their processes, and their products and test accordingly rather than hiding behind blanket finished product testing.
Otherwise the vista of 100% sterile manufacture in place of Non Sterile Medicines will eventually make more sense from a safety, quality and eventually economic perspective , as added costs will always get passed on to the to the public.

Given the current debate around testing of BCC in Aqueous products , one might also ask whether manufacturer's need to exert greater control with regard to product usage conditions, where it may be necessary in the future to restrict availability of certain product types or formats to certain patient categories or dispensing environments, where it is considered that risk of contamination and infection is too high, given the practices and susceptibility of the patient population involved.

Anyway - just an initial thought for the day.

A28: The USP is too precious in writing <60> and should defer to <1231>! The USP Microbiology Expert Committee is not responsible for <1231> but the FDA was supportive of <60> in screening aqueous non-sterile drug products for Bcc.

A29: Agree on many of the things you wrote, but not on using a differential medium for water counting. I am not talking about product control, in which it is correct to do a pre-enrichment for the reasons indicated by you. I speak only for water control as process (or raw material) control. As you know, in many cases numerical controls are performed by carrying out counts in selective media for various types of contaminants (see ISO standards). For example, coliforms and faecal streptococci in water for human use, are enumerated directly by filtration.

A30: You are quite right that methodologies using selective and differential agents for water are commonplace – but “test context” is key, i.e. - type of water being tested, test objective and how the result outcome is interpreted and used to influence quality management decisions.

In my experience - primary testing using selective media is frequently used for Raw, Potable and Pre-Treatment Stage waters - where numbers of the target organism are relatively high and the relative loss of method sensitivity due to the selective process adopted, may not significantly influence the outcome. In addition, many of the selective tests are based on the principle of Presence or Absence (P/A) or allow for some level of semi-quantitative or statistical estimate (MPN etc).

Quantitative recoveries are more usually anticipated with the use of non-selective general growth media.

As always is the case – it depends.
Personally, I wouldn’t recommend the use of direct selective (and to lesser extent differential) media for enumeration of specified microbial types from High Purity Water Systems (HPWS) such as PW etc,
but if such an approach works effectively for you - well and good.
Good Qualification and Period Verification data will always trump opinion.

Sure, we can isolate typical water GNBs from HPWS directly on selective media - recovery per se is not the issue, the question is whether we can demonstrate that recovery is equivalent to that of non-selective media and whether recovery efficiency is reproducible. Qualification or verification can be a challenge or at least require more effort of work and performance monitoring input than that required to subculture and presumptively ID a small number of isolates from plates (R2A, PCA etc) or associated membrane filters.

That said, on occasions I have seen effective use of parallel testing of water systems using both non-selective and direct selective methods under non-routine conditions, when implementing Corrective Actions in response to quality excursions or system design performance issues involving a specific contaminant (usually an "Objectionable", as locally defined).
In most of these cases the methods used were P/A and involved enrichment methods and non -routine (higher frequency, higher volume) sampling programs.

Anyway, in the end It all depends on what works for you and what maintains or improves your Quality Outcomes.

Environmental monitoring incubation Condition

Is there any reference document or guideline for incubation condition for environmental monitoring plate i.e first two days 30-35 and after 20-25 for three days. Mostly guidelines provide only limits but not incubation condition. Please suggest.

A1: There is no one set incubation scheme. There is data showing 20–25 first followed by 30-35 and vice versa. If you’d like one plate and two incubation conditions, you’ll need to show equivalent recoveries of the scheme you choose compared to the recoveries you get at different temps or different media with different temps. There really is no “one way”.

A2: Your incubation conditions will be dependent on the type of manufacturing (pharmaceutical, medical device, consumer care, etc.) for and the type of test (surface, viable air, etc.). Standards will state different conditions depending on the manufacturing. For example, standards like USP <797 (Sterile Compounding) or USP <795> (Nonsterile Compounding).

Example USP <797> proposed revision on Active Air Sampling
1. Two samples may be collected for each sample location and incubated concurrently.

1. Both samples could be TSA or one sample could be TSA and the other fungal media (e.g., malt extract agar [MEA] or sabouraud dextrose agar [SDA]).
2. Incubate each sample in a separate incubator. Incubate one sample at 30°–35° for no less than 48 h, and incubate the other sample at 20°– 25° for no less than 5 days. If fungal media are used as one of the samples, incubate the fungal media sample at 20°–25° for no less than 5 days.

Another Example USP <1116> Microbiological Control and Monitoring of Aseptic Environments

1. Selection of Culture Conditions
    * For general microbiological growth media such as SCDM, incubation temperatures in the ranges of approximately 20°–35° have been used with an incubation time of not less than 72 hours.

USP website has numerous standards and proposed revisions. Hope this helps!

A3: There is mention in <1116> in the section for culture conditions relating to the topic of using single plates at both temperatures, that reads "Incubating at the lower temperatures first may compromise the recovery of Gram-positive cocci that are important because they associated with humans" so would relate to EM/PM monitoring.

Maldi-TOF identification system

I have a colleague who is having trouble with obtaining results following its introduction to their lab. The only experience I have is from what I've read on the subject and from what they have also told me about their issues. I'm looking for some help or ideas that I can pass on please. Apologies for the large amount of information.

They seem to be getting quite a lot of results that are not able to be identified to the level of certainty/log score. In order to fully identify the isolates, they are then having to send these away for genetic ID with MicroSEQ. The result they then get is not on the Maldi database and is causing frustration as they believed that the Maldi library is one of the largest and certainly larger than their Vitek they previously used.
Q1. How comparable are results from the two systems and if the MicroSEQ provides a result from its manufacture validated library (not the EMBL database)? (e.g. if they get a result from the MicroSEQ could it be inaccurate and shouldn't be compared to Maldi database?
Q2. If an acceptable high confidence result is not achieved but a lower score, can the low confidence identification be used to report the result to Genus level only? Or due to how it works/compares to its database could the genus result be completely wrong at that stage (e.g. would multiple low confidence results for the same organism analysed provide different genus results)?
Ability to use a lower (and acceptably validated) confidence genus result would really help them.

Also, purified water isolated organisms seem to also be consistently problematic and routinely need to go through to extraction and even then are not being identified to high confidence. I believe that fresh culture (18-24hrs) is not always required, but can be preferred as the culture should be actively growing to give a result, you can use isolates that may have been incubated for 5 days or more. However, they have tried processing older and fresh isolates and multiple times.
Q3. Is there anything that can be done to the sample prep for water isolated organisms to try and obtain a better culture?

They were also under the impression that for general bacteria that the majority of results should be obtained from a direct transfer, however a few percent may require extended direct. They are needing to perform extended direct on approximately 40% and then extraction on about 10% of all samples. Again, their understanding is that extraction is really only for moulds or certain bacteria with difficult membranes. Q4. What are peoples' thoughts on this level of results and needing to routinely do full extractions on bacteria isolates?

Any information or experience that you can provide would be greatly appreciated.

A1: Q1. How comparable are results from the two systems and if the MicroSEQ provides a result from its manufacture validated library (not the EMBL database)? (e.g. if they get a result from the MicroSEQ could it be inaccurate and shouldn't be compared to Maldi database?

Different ID technologies have limitations with some genera and all have database limitations. The majority of species most frequently isolated occur over and over again. Around 20 bacteria represent 50% of the isolates See Guilfoyle and Cundell (2022). If a bacterial isolate is not the MALDI TOF database it can be identified by 16s rRNA base sequencing and then added to a customized database.

Q2. If an acceptable high confidence result is not achieved but a lower score, can the low confidence identification be used to report the result to Genus level only?

Sure why not especially if the isolate is detected at low frequency.

Q3. Is there anything that can be done to the sample prep for water isolated organisms to try and obtain a better culture?

Identifying the bacterial isolates directly from the primary isolation plate saves time and money. There may be a trade-off between incubation time and identification. Also you need to decide if the extraction procedure will be your default procedure. Subculture water isolates especially the transition from R2A to TSA can be a challenge.

Q4. What are peoples' thoughts on this level of results and needing to routinely do full extractions on bacteria isolates?

This may be predicated on your workload.

A2: You hit the nail on the head with the statement, "the result they then get is not on the Maldi database."

All automated and rapid microbial ID systems are only as robust as the database or library the system contains. If the isolate is not in the database, then the result is no ID or a low similarity index/confidence level.

Many companies using MALDI experience the same thing; no or low confidence ID is followed up with another method, such as 16S rDNA analysis.

So my question would be, after the 16S result, does the lab add the Genus and species into the MALDI database so that the MALDI can ID the same species if it is found again?

Was there a difference in the fresh versus older cultures for a correct ID in the water samples (assuming the isolate is in the MALDI database)?

Finally, most companies will not perform the extraction procedure for bacterial ID's.

A3: I think that the plan will be to try and add the new ID's to their system, however they need to look into how complicated this is and what type of validation needs to be performed. As far as I'm aware they may

need to update their software with an additional package to allow this to be performed with full traceability and validation.
Is there any suggestions you may have about the complexity and expectations for adding their own organisms as this is a GMP pharmaceutical manufacturing site with US,EP and other ROW regulatory bodies that inspect.

For old vs new. They do regularly find that a fresh culture will go on to provide a reportable result for both water and general EM isolates. I'm not sure of the amount that would then come off direct, extended direct or extraction.

Do you have any thoughts on the ability or not to be confident in the lower confidence genus results and able to report them?

A4: With your comment that they could accept the low confidence result, that sounds like promising news. However, the concern is would low level results have been sufficiently assessed/validated to be provide the same consistent genus result. With how the Maldi provides its result from comparing points to the database, is the genus provided for a low confidence result accurate or could multiple genus be given as possible options as i believe that the final result lists the top 10 or 20 (not sure) results that it thinks could match under the actual best fit.

With regards to the level of extractions required for bacteria, they were under the impression that approximately only 1-2% would possibly only need this with almost all bacteria coming off from direct/ extended direct methods. However, they are having to do this quite regularly so wondering if there may be method issues or something possibly influencing not getting a result form direct or extended.

A5: Depending on the MALDI system, there should be instructions on how to add a Genus and species to the database, linked to the MALDI signature. If the software allows this, you should be able to validate adding the new organism and then demonstrating a correct ID when the organism is subsequently processed in the system again.

You always have the option of reporting a Genus only result, but that limits the power of the MALDI platform.

A6: The comments on library building will differ based on which system is being used as they are fundamentally different. I'm aware of 2 widely commercially available MALDI systems.

One system has independent reference spectra based on multiple measurements from a single defined strain. This is a good way to respect the normal intraspecies diversity which can lead to no identifications on MALDI systems. Preparing library updates is a “straightforward” (more on this below) process of adding / replacing / removing single strain library entries as needed for a well-maintained and curated database.

The other system divides the reference peaks into defined weighted bins for the reference species. Due to the combining and averaging of different strains’ spectra at the reference species level, strain nuances can be lost, and library updates are much more complicated as the weight for all the bins may change with each entry, thus the whole library can be affected.

With regards to how complex or straightforward it is to add, remove, or modify an entry to a MALDI library – including new entries from strains or species that were identified through sequencing – it should be taken into account that, as a regulated industry, the user should have a quality system purposely designed to ensure that laboratory processes, computerized systems, and equipment, as well as the reference libraries that are used to generate test results, are all adequately maintained in a state of control.

Modifications to MALDI libraries require the user to maintain its validated state using a quality system that meets industry regulations and compliance. This should include routinely performing library re-qualifications to ensure accuracy and consistency of identifications.

The effort required to do this will vary by instrument manufacturer. Moreover, the procedure to include a certain entry involves several additional processing steps and often requires more time and effort than people expect. Data generation and analysis, and the quality checks that go with that, are time consuming.

It is also noteworthy that not only are new species being routinely encountered that are not represented in MALDI reference libraries, but also different microorganisms' strains can generate quite different MALDI spectra. Similarly, different growth stages for fungal samples have been demonstrated to generate significantly different MALDI spectra. Including these unique entries into a library increases the possibility of generating higher scores and an ID. This is only possible for one of the commercial platforms.

These novel species and this variability are the reason a user can continuously add entries into their database, but there will always be gaps that need filling – and it is a significant effort in labor, time, money, and validation burden. Basically, the users could add a certain entry to their database, but they won't be never 100% sure they will be able to get a good score again in the future, if a different strain will be sampled, or a different growing stage will be used.

A7: With regards to how complex is adding a new entry to the MALDI library from sequencing name/result, it should be taken into account that, as pharma industry, they should have a quality system purposely designed to ensure that laboratory processes, computerized systems, and equipment, as well as the reference libraries that are used to generate test results, are all adequately maintained in a state of control.

Adding an entry to the MALDI library requires maintaining it validated, which includes features like 1) updating the names of entries to reflect taxonomic changes and accuracy of names, 2) routinely performing library re-qualifications to ensure accuracy and consistency of identifications and 3) maintaining a validated state of the library using a quality system that meets industry regulations and compliance.

Moreover, the procedure to include a certain entry includes several additional steps and requires more time than expected. The MSP generated by running a whole plate, must be run against the original spectra to see if it provides an ID, and against the whole library to ensure specificity. Then, some of the generated spectra are run against this MSP and the whole library again.

It is also noteworthy that different microorganisms strains can generate quite different MALDI spectra. Similarly, different growth stages for fungal samples have been demonstrated to generate different MALDI spectra, and including those MSP into the library increases the possibility to generate higher scores and an ID. For this reason, they could add a certain entry to their database, but they won't be never 100% sure they will be able to get a good score again in the future, if a different strain will be sampled, or a different growing stage will be used.

Analyst requalification criteria

Kindly provide insight on microbiologist qualification criteria (acceptance limit) in microbiology tests like-MLT(TAMC,TYMC), water testing. spore enumeration, bioburden test etc.

Swabs

Is there any use in performing swabs in a ClassA/ISO 5 room, with a limit of no CFM, as we know the recovery for swabs is well below 100%. Any negative result is likely to be unreliable and wont demonstrate cleanliness of surface.

From memory ISO 1737 required validation of swabs only with Bacillus spores. Is this acceptable? Is there any minimum recovery of challenge bacteria needed so that swab validation conformed?

Method verification for a ongoing product with increase active ingredient concentration

Need your opinion on this We are manufacturing a dye based tablet in a strength of less than 100 mg. Recovery study for bacteria, fungal species for enumeration test and pathogen detection is already performed and method is validated too as per USP <61> and <62>.

We are going to manufacture the same product with increased concentration of 200 mg or 500 mg. The product has no antimicrobial properties. My concern is about recovery study. Do we need to perform the recovery studies (Method Verification) for new strength?

A1: Perform method suitability at the highest level and this way you bracket all concentrations (assuming everything else remains identical). Michael

A2: Since you already have established successful recovery at 100 mg strength, you should at minimum perform for 500 mg strength so you can bracket in 200 mg dose. This is provided the 500 mg strength shows adequate recovery at the same dilution in same media.

Using FTM (Fluid Thioglycolate Media) and TSB (Tryptic Soy Broth) as enrichment media

Would like to check in for general culture contamination screening, is there a guideline on how long test subjects should be inoculated in FTM (Anaerobic) and TSB (Aerobic) before subculturing on solid media, TSA/TSA-SB (Anaerobic) and TSA (Aerobic) respectively? I am trying to improve the contamination screening process in which samples that are not suitable to be plated directly to solid media for e.g resins, anti-foam.

Oceanobacillus Kimchii

Has anyone seen or heard of multiple recoveries of Oceanobacillus kimchii from surface monitoring of an aseptic processing area? I am dealing with a situation now of multiple low level hits for an organism that, as far as I have discovered, was isolated from the ethnic fermented food Kimchi and has also been isolated from sea sponges. It is a mild to moderate halophile and I am surprised to see this being isolated from the APA. Any help is greatly appreciated.

A1: Have you reviewed your personnel monitoring data? How strong is your hygiene program?

Have you interviewed your operators? Maybe someone started at at-home kimchi making hobby? Maybe someone started growing sea-sponges (the sea monkeys of 2022!) in a basement aquarium (not the weirdest thing to happen in NJ).

Have you evaluated your disinfection program as well? Are you making your disinfectants from concentrate or buying RTU formulations?

Has any new equipment been moved into the cleanroom? How about new consumables or devices transferred into the warehouse? New carts? How long have you been recovering this isolate and is there a potential vector (follow the breadcrumbs!)

Are the recoveries of the isolate on the facility surfaces (walls, floors) or from areas that personnel may contact?

Lots of questions I would ask in an investigation (shameless plug for PDA TR88 right here!).

Welcome to the world of finding Waldo in your cleanroom!

A2: I have seen this recovery on occasion at 2 client sites in the Boston area over the last several years in EM sample plates, primarily surface samples. Both clients had aseptic processing areas, but I can't recall if

it was ever isolated from Grade A areas. It is a spore former but neither client sent out samples for disinfectant efficacy testing.

A3: My lab has identified microorganisms from the environments of a wide variety of medical device and pharmaceutical manufacturers. I have seen this organism pop up a handful of times, though it isn't very common. It is a spore-former, and thus requires a sporicidal disinfectant to manage. If you are starting to see multiple recoveries of this and similar spore formers, perhaps an assessment of cleaning practices would be a good place to start.

I wouldn't get too hung up on the kimchi or the sea sponge aspect. Many bacterial names reflect the source of the microorganism at the time it was first described. However, this doesn't necessarily mean that it is the ONLY place the microbe may be found. Environmental spore-formers like this can enter a controlled environment via anything that is coming in from the outside (humans, supplies, packaging, equipment, etc.). Even if a conclusive source of the organisms cannot be found, you can still evaluate your manufacturing and sanitization processes and take action to respond to the new trend.

A4: Here are some additional details:
We are aware that O. Kimchii is a spore former, and the APA is cleaned using SporKlenz every 7 days. Most of the 5 recoveries were from the floor of different rooms within the APA over a 6 month period. The CMO has not recovered the organism from any personnel monitoring so far. While they do not have a definitive root cause, they have proposed to disinfect the wheels of the carts used to move equipment and consumables into the APA with 6% H2O2.

Gram negative rods in a clean room

When Gram negative rods are isolated we can look at the species (Pseuds and related) and usually categorise them as to where they may have come from e.g water borne/drains
My conundrum is as follows,
if you use water in the area for cleaning/making disinfectants/rinse (e.g. WFI or 80oC Purified) and this is routinely tested and no Gram negatives ever isolated, where are the water borne Gram negative rods coming from?

How are we isolating them from floor surface monitoring samples? this is usually monitoring to show that cleaning and disinfection is under control.

Are we assuming that a sink drain could be the source and managing to get tracked through the cleanroom somehow and have low counts (1 to <5cfu) survive a disinfection regime.

A1: Look at all hoses and water sprayers, cleaning supplies (buckets, brushes), shop vacs, etc. look at everyplace water can be used. Look at all unit operations that are cleaned for areas which never dry or trap water that can stagnate. Good starting points.

A2: We will be looking at the areas you have suggested.

However, how have they been introduced into these possible areas (hoses, buckets, etc) if the water in use is not showing any Gram negative rods?

Are they actually in the hot purified water or WFI but we just haven't detected them? or are they being introduced via personnel/material/equipment as an environmental origin that has been transiently brought into the areas and we are just used to thinking of them as being from a water source but in reality that may not be the case?

A3: Good questions [name redacted]. Do any of these locations allow for stagnant water to remain u disturbed? If so you can expect microbial growth. If your WFI system has deadlegs or inadequately maintained unit operations then this could be a contamination point of concern. And if the hot PW system

has any location that cannot maintain the heat, then this is another potential issue. As for not seeing gram negatives, this may depend on whether your current test methods are sensitive enough or whether you are sampling correctly and in the right locations. We have found this to be the case with many of our clients with water system contamination problems.

A4: [name redacted] I've seen firms that would wash the outside of their tanks in a Grade D washroom with a garden hose balled up and stuck on a wall. They'd connect this garden hose to a sink with tap water. Regardless of what your procedure says, if they're required to be using hot WFI, ensure / verify that they are actually DOING that during all shifts of operation.

A5: And if you Are using hot WFI, why in the world wouldn't you spend the money on a legitimate pharmaceutical grade hose? The plastic inside garden hoses are prone to breaking down, creating thousands of places for microbes to hide.

In any event, is the hose part of a validated cleaning program?

A6: Sometimes I also observed the same issue particularly when there is CIP-SIP in the clean rooms but couldn't find anything. During normal' day's there is no microbial recovery.

Is there any probability that these gm negative bugs are present in the area and growing only when there is water in the area??

A7: I have seen lots of great responses to your question. Look for places where non-purified or WFI is being used and sneaking into your process. Also, look for sources of standing water. Remember that, even when you are using pharma grade water, it may not be sterile. You are probably testing 100 or 200 mL of water and the contamination rate may be a lot lower, but when many, many liters are used for cleaning, or other operations there may, in fact, be GNR's present. Also, look at the cleaning procedures utilized. I have been involved, at least twice where the cleaning led to water getting into the back of equipment and only found when the equipment was broken apart to do some type of maintenance. There was no idea how long the water had been getting behind the gaskets, but it was there and was a GNR soup by that point.

validation of automated colony counter

In a validation study, I want to know, ¿What is the acceptance criteria for the relative standard deviation when comparing counting using automated equipment and manual counting?

What is the analytical reference for this criteria?

A1: I believe different analysts would be expected to be within +/- 10% around 85% of the time.

See
*Jones, D. and T. Cundell *Method Verification Requirements for an Advanced Imaging System for Microbial Plate Count Enumeration *PDA J Pharm Sci Technol* 72(2):199-212 2018

Disinfectant Residue

The subject of disinfectant residue seems to be creeping into discussions recently. Mainly from disinfectant suppliers. In essence it is being suggested the residue will continue to act on a surface after the initial disinfectant wet contact time, when the surface is dry.

Though I accept residue may have some biocidal or even biostatic activity in some circumstances. I can't envisage this will ever become a valid tool for surface disinfection in a PHARMA setting.

In the unlikely event it was possible to apply disinfectant in such a way that an even active layer of residue was present on a surface.
Validating the biocidal activity seems to me to be almost impossible.

Expert views would be very welcome

A1: Your disinfectant program should include cleaning, disinfection, and residue removal. The effectiveness of this program would be supported by laboratory studies and routine environmental monitoring.

Disinfectant residues continuing to act is a bad idea as residue build-up may lead to reduced application effectiveness and potential for chemical adulteration of the your products.

A2: The subject of residual kill value will be mute with the introduction of EU Annex 1 Rev13.
Compliance with the new paradigm separation of "Cleaning" from “Disinfection", will require all companies with GMP areas to re-write their SOP's for those areas.
New EU GMP Annex 1 Revision states:
4.24 The Cleaning process, prior to the Disinfection process, is Essential.
. Any residues that remain may inhibit the effectiveness of the Decontamination process
4.36 For Disinfection to be effective, prior cleaning to remove surface contamination should be performed
5.4 The Cleaning process should be Validated to:
1. Remove any residue or debris that would detrimentally impact the effectiveness of the disinfecting agent used.
2. Minimize chemical, microbial and particulate contamination of the product during the process and prior to disinfection.

A3: [name redacted], I would ask every supplier that suggests this to back up their claims with actual data. Otherwise, they are misinformed or outright lying.

A4: I agree with [name redacted] (the stars must have aligned!). This is pure nonsense.

There is no practical way for disinfectant residue to be effective, nor has this ever been demonstrated in any peer reviewed articles.

The ONLY way this is might be barely or remotely “plausible”, would be if there is enough humidity/ moisture/water in the area to resuspend/rehydrate the residues (and no, that isn’t feasible either). If that ever happens, you’ll have bigger issues to deal with.

A5: I agree with you about not being able to apply an even layer to validate residue. One approach that you could try to validate though is as done for test for antimicrobial activity of surfaces like in JIS Z 2801 and/or ISO 22196.

You're probably aware there are residue removers on the market, though I have no experience with these. Hence the recommendation from the vendor?

A6: The first question is how does the disinfectant kill the microorganism. I would have to interact with the cell wall because there is not liquid to transport the chemical into the cell.

In my experience one the disinfectant dries on the surface it can increase the particle load in the room. It is released every time someone disturbes the surface, usually by hitting the walls or hitting or rubbing a surface in the case of equipment. I have demonstrate this is multiple plants that indicated that they do not remove the disinfectant after the exposure period. You just hit the wall and watch the room particle counter alarms go off.

So even if the anti microbial agent residue is effective, it causes other problems.

I do not recommend the leaving of disinfectant on surfaces at the end of the validated exposure time.

A7: It is true that there are some disinfectant products that claim to have residual antimicrobial activity, meaning that they continue to be effective after they have dried for a period of time after being applied to a hard, non-porous surface. In addition, you will see residual antimicrobial claims for antimicrobial handwashes (e.g., with Chlorhexidine digluconate or povidone-iodine as the active ingredient) or with disinfectant products that are used in a hospital environment or other health-care settings. You will also see this residual claim on those disinfectant products that are used on food contact surfaces that contain either Calcium or Sodium hypochlorite as the active ingredient. It should be noted that Isopropanol or Ethanol does not have residual antimicrobial activity. To demonstrate residual activity of a disinfectant, see E.P.A test Protocol #01-1A Protocol for Residual Self-Sanitizing Activity of Dried Chemical Residues on Hard, Non-porous Surfaces. For antimicrobial handwashes for demonstrating residual antimicrobial activity, see ASTM E2752-Standard Guide for Evaluation of Residual Effectiveness of Antibacterial Personal Cleansing Products.

From my perspective, it would be inappropriate to leave a chemical residue from either a disinfectant or sanitizer on an equipment surface that is used to in a pharmaceutical manufacturing plant for finished products for antimicrobial residue activity because some of the active ingredients in these disinfectants or sanitizer are toxic. For this reason, rinsing of chemical residues of disinfectants or sanitizers are verified during validation of the chemical sanitization process by conducting TOC testing of purified water rinse water samples to prevent this occurrence.

If a disinfectant or sanitizer company is indicating to use their products for reducing the microbial bioburden of floor surfaces in a non-sterile pharmaceutical manufacturing facility by leaving a residue of active ingredient, I might consider it after conducting some environmental microbial testing of the floors. However, I would not accept anything from a disinfectant or sanitizer manufacturer as gospel without thinking about it and conducting some testing. In addition, I would like to see the product label of the disinfectant or sanitizer product that would have this claim listed since the E.P.A. during the registration process would need to have reviewed the test data and approved this particular claim for residue antimicrobial activity before the product is ever sold.

A8: ome types of disinfectant residues when hydrated do appear to impart some effects, based on some synergistic effects we have observed in some of our studies. Further, this is also suggested by the importance of neutralizers on RODAC plates. The purpose of these neutralizers is to minimize antimicrobial effects from disinfectant residues which may be picked up from the RODAC contact with cleanroom surfaces that have been treated with disinfectants. However, that alone would not mean the residues are uniform or validatable. Besides, residues in cleanrooms may come from many other sources including personnel, raw materials, products, and equipment. Therefore, a cleaning and disinfection program should also include periodic rinsing for residue removal. Water for injection, 70% isopropanol and in some cases low concentrations of a neutral cleaner will all work well for periodic residue removal.

A9: I think it's clear the residue discussion has little value in a pharma setting. CEN and AOAC disinfectant testing is after all carried out in wet conditions too. So with no recognized methodology or standards for the anti microbial effect of residue; the argument for a residue effect remains unfounded.

Sterility Testing of Cephalosporins

My company is trying to perform B/F testing of cephalosporins per USP <71>.
We have added β-lactamase to the media (TSB and FTM) at several different concentrations but have not been able to neutralize the product.
This product also contains cottonseed oil. The product would not filter undiluted.

Any advice would be appreciated!

A1: I found in many cephalosprin formulations, that adding substantially more rinse works best. We used 500 mL to 1 L rinses to extinguish the drug from the filter worked.

A2: Have you tried the specific membranes that are combined with the steritest for fatty substances? For example: The Steritest ™ NEO device for veterinary cream, ointment and injectable solvents with green canister is designed to test products dissolved in solvents such as isopropyl myrysate.

A3: If you perform the test as per USP then please assure your inoculums size(which will not be more than 100cfu/ml) and which type of inoculums you used. Then try the neutralizing agent with your hand practice, it means your sample volume which you drew and apply different concentration of neutralizing agent.

Loop-Mediated Isothermal Amplication (LAMP) Assay for Detecting Bccc

Does anyone have experience in using the Loop Mediated Isothermal Amplification (LAMP) Assay for detecting the presence of Bcc in purified water samples? I see that an article on this particular assay had been published in a journal called Pathogens in August of 2021 (https://doi.org/10.3390/pathogens10091071). It seems that some of the authors of this article are with the FDA. Based upon an internet search that I had conducted on this assay, I do not see the availability of a commercial kit to perform this Bcc detection assay. It seems that you have to order the reagents and primers separately and combine them together to make your own kit to perform this assay. From my perspective, I see a lot of cGMP issues with this approach.

My reason for asking is that it seems that you are able to have rapid detection of the presence of Bcc in enrichment samples after incubation without using BCSA by using this ribB-based colorimetric LAMP assay. This assay could be an advantage over using BCSA selective/differential agar in the future for detecting the presence of Bcc isolates in a test sample if a LAMP commercial kit was ever available.

A1: Hi Don. I know there was talk of using LAMP for GMO foods because it could be done at the barge upon loading of the corn, in the field…as one example. That was a collaboration between private companies and a university…but I don't think anything ever came out of it. They thought it would work because there was plenty of DNA to isolate, eliminating the need for enrichment. What caught my eye in this paper was this statement:

"Many of the samples currently analyzed by USP (60) (microbiological examination of nonsterile products—Tests for Burkholderia cepacia complex) were plated on Burkholderia Cepacia Selective Agar (BCSA; Remel, Lenexa, KS, USA) [8], which required a median time of 16.5 days for the first positive culture from contaminated liquid docusate [29]."

What does this say about our current USP method?!?!

A2: I just posted this in our rapid methods LinkedIn group (https://www.linkedin.com/groups/2884229/).

I am not aware of a commercial system using LAMP for BCC detection. But this would be a great advantage over USP 60, assuming it can be validated as being non-inferior or equivalent, in terms of time to result.

Propylene Glycol Endotoxin MVD

I am looking for help in calculating the MVD of injectable grade propylene glycol (liquid). The spec that we were given according to ChP is 0.012 EU/mg. I haven't seen a liquid with a specification in EU/mg previously, so I was trying to figure out if a concentration needs to be considered in this calculation?

USP 71 System Suitability

In our USP 71 Sterility System Suitability testing, our product and positive controls (15 mL TSB media) for Aspergillius brasilaiensis are incubated at 20-25C for the requred maximum 5 days. With Aspergillus and other molds sometimes taking 5-7 days to grow, why is this organism the same as all the other required USP 71 organism when it takes potentailly longer to grow?

As a history question, has the maximum incuabtion time of 5 days ever been longer in the past for Aspergillus or the other organisms?

Any insights would be helpful.

A1: For Growth promotion and method suitability, I don't believe the incubation time has ever changed. Are you able to see growth in 5 days with no product? As far as the sterility test itself, there have been multiple changes since its introduction into the British Pharmacopeia in 1932. When introduced, it was controversial due to sampling, temperatures, accuracy, inhibition of media, etc. Lots of studies were done to determine the current method of 14 days, 2 temperatures, 2 media. I have a few papers that talk about the history of the sterility test and some are from the 1950's! We're working with as many knowns as we can but as any microbiologist knows, sometimes the bugs don't cooperate! Here's a good paper that talks about incbuation conditions with the sterility test in case you wanted it:

TECHNOLOGY /APPLICATIONS
The Incubation Period in Sterility Testing
HELEN BATHGATE, DAVID LAZZARI, HELEN CAMERON and DAVID MCKAY1'
Therapeutic Goods Administration Laboratories, Canberra, Australia

Qualification of pure steam generation system

Having question regarding Pure steam Qualification. We have introduced new purified water system and Water for injection system (Generation and distribution) . Phase I study of the PW system is under process and after qualification of PW system , qualification for WFI system will be perfromed As we know that PW is used as feed water for Pure steam generation also and PW system is already will be qualified so is there any requirement to perform Phase wise monitoring (Phase & Phase II) for Pure steam system Please do share if there is any technical guidance available with you Your quick response will be highly appreciated.

USP <71> for Viral Vector Manufacturing

What's the best way to complete USP<71> method suitability for viral vector products when your final product is such a small yield? Any guidance is appreciated.

A1: You may want to consider an alternative approach such as USP <1071> or EP 2.6.27.

A2: Have you read the "Amendments to Sterility Test Requirements for Biological Products"? There should be some useful information in that document. It was published by FDA in 2012.

A3: This also depends on the volume of finished product you intend to test. Can you justify using the 1% rule? If so, method suitability would be performed using this amount. Otherwise, you would follow the requirements in USP 71.

Microbial quality of antibiotics

I would like to request information about microbial quality of antibiotics. I was unable to find any information about them. I am not talking about the antibiotic assay o "potency assay", I want to focus on the tamc, tymc and pathogen detection in oral antibiotics.

I know that in antibiotic drugs there will not be microbial growth but you have to check it, to perform the suitability method, etc. So, how is it performed?

Although, I would find a neutralizing broth or strategy to my tetracycline and I would be able to perform the normal microbial assays (TAMC, TYMC, etc) If the bacterial count would be to high (not complies with specification) I would reject the batch?
What do Normative or guidelines say?

A1: I'm not sure as to the reason as to why you would want to conduct microbial limits testing on an antibiotic powder as a raw ingredient. I would think that a conducted risk assessment would show that antibiotic powders as a raw ingredient would be hostile to the survivability of non-spore forming bacteria.

Instead of conducting a pour plate method for Total Aerobic Plate Count and Total Yeast and Mold Count in which antibiotic neutralizers would need to be used, I would suggest that the antibiotic powder first be dissolved in sterile deionized water and then passed thru a membrane filter that is rinsed to determine the microbial content of the sample. For enrichment, I would place the rinsed membrane filter in which the dissolved antibiotic powder that has been passed through would be placed directly into enrichment broth.

If membrane filtration doesn't work during your suitability testing, I believe that Iron ions that are able to neutralize tetracycline could be used in the neutralizing diluent. See the following reference:

The neutralization of antibiotic action by metallic cations and iron chelators. A. A. Miles, J. P. Maskell Journal of Antimicrobial Chemotherapy, Volume 17, Issue 4, April 1986, Pages 481–487, https://doi.org/10.1093/jac/17.4.481

For microbial test specifications, I would think that you would use the same specifications in the USP for oral products if the antibiotic finished product is a non-sterile oral product.

A2: Alba, a starting point would be the general microbiological requirements for pharmaceutical ingredients in USP <1112>. You would take a risk-based approach in terms of antibiotic synthesis, testing history, contribution to the dosage form, product formation and manufacturing process, etc to justify if routine testing was necessary.

A3: Thank you for your answer and the information related to neutralization of antibiotics.
The reason for conducting microbial limit tests in products that contain antibiotics is to count or elucidate the presence of fungal or non-sporing bacteria.
It is obvious that most bacteria cannot grow in that kind of product.

I try neutralization by dilution but there is no growth of the pharmacopeia strains even in the 1/1000000 dilution. In my opinion, dilution is not the best way because if it is necessary to reach a high dilution I am not testing the product, I am trying the buffer used for the dilution (of course I will see growth there).

The problem lies in the solubility of my products. It is impossible to filter them, so I have no choice or I neutralize the antibiotic of the formulation (gentamicine or tetracycline) or I will consider that my product is safe and is not likely to be contaminated.

But, What does normative say about antibiotics? I am unable to find anything.

A4: It is my understanding that Tetracycline was soluble in both water and ethanol. As far as a chemical neutralizer, most heavy metals such as Al, Calcium, Iron ions will neutralize tetracycline by preventing the absorption of tetracycline by the human gut. I do not know of any other ingredients that will chemically neutralize the antimicrobial activity of tetracycline that could’ve been used in plate count diluent. Based upon your data, I would say that dilution is not the answer. In your suitability testing, are you having issues with all of the test organisms? If you are having issues with the recovery for all of the suitability test organisms, this data could be used as part of your risk assessment in not conducting microbial content testing of tetracycline by showing hostility to microorganisms.

For additional supporting material for not to conduct microbial limits testing, I would suggest that you check out the manufacturing process. They might be using chemicals that might render the material to be free of organisms. I believe that butanol was used in the manufacturing process at one time. I will admit that it has been a long time in which I was involved in the manufacturing of tetracycline, but I do not remember ever conducting microbial limits on the raw material powder.

You will need to realize that not every raw ingredient used in a non-sterile product formulation would be susceptible to microbial contamination and there is no need to conduct testing in it.

Current coliforms method

Kindly suggest coliform test (qualitative) as per USP guidelines.

A1: USP refers to city rules that refer to other EPA approved methods. ReadyCult would be an option that's qualitative and on the list of EPA approved methods. I'm not sure of the requirements in other countries.

Burkholderia water testing

I want to test purified water (PW) and water for injection (WFI,not-sterile) according to USP<60>. Due to the low total counts i test 100 or 200 mL of samples. Should this be pre-incubated in TSB at 1:10 dilution, before transfer to BCSA plate, as suggested by USP <60>? This poses a technical difficulty.

USP AET Microbial Test Organisms

I have a question for the group. In conducting USP 51-AET, the recommended challenge test organisms for usage are as follows:

Ps. aeruginosa ATCC 9027
E. coli ATCC 8739
S. aureus ATCC 6538
C. albicans ATCC 10231
brasiliensis ATCC 16404

My question as to why each of the above species and strains has been selected for usage to conduct preservative challenge testing in USP 51? From time to time, I have heard a lot of different reasons from people saying that these species had been selected because each of them is a pathogen, or each represents a microbial physiology. I always felt somewhat uncomfortable with these 2 explanations as to why each of these species had been selected for usage in conducting challenge testing. In the Introduction section of USP Chapter 51, it is stated that challenge organisms are generally based on likely contaminants to a drug product while considering its physical attributes, formulation and intended use. I considered this justification for the microbial species in USP Chapter 51 to be somewhat weak.

When I was taught how to perform challenge testing, I was told that each of these microbial species and strains had been selected for conducting challenge testing because they are indicator organisms for groups of organisms that could contaminate products during consumer usage. For example, Ps. aeruginosa had been selected to represent all of the possible non-fermentative Gram-negative bacteria contaminants, E. coli was selected to represent all of the possible fermentative Gram-negative bacteria contaminants, Staphylococcus aureus was selected to represent all of the possible Catalase-positive Gram-positive cocci contaminants, Candida albicans ATCC 10231 was selected to represent all of the possible yeast contaminants, and Aspergillus brasiliensis ATCC 16404 was selected to represent all of the possible mold contaminants. As for each of these ATCC strain numbers, each of them is not considered to be type strains for the species. I'm O.K. with them not being type strains.

Does anyone have a better explanation as to why these microbial species and strains had been selected for usage in conducting challenge testing from what I had been taught or is in the USP? From my perspective, the usage of these species as indicator organisms for groups of organisms is a more realistic explanation as to why they are being used to conduct a challenge test.

A1: Come to mind;
Could these microorganisms be common in hospitals and clean rooms? For
example, S. aureus is usually found in the nose of humans, E. coli in human feces…as

A2: bacteria and the Candida are all from clinical sources and 16404 is from blueberry. Based on submission dates - the oldest is 6538, approaching a century from original isolation, and the most recent is 16404, think submitted back in the 1960's.
Scott Suttons article claimed these were based on recommendation of a comm. of the Pharm Manuf Assoc. that "... concluded that the types of test organisms should be those that were found to contaminate theproduct—either through use or introduced with the rawmaterials." http://www.microbiologynetwork.com/content/file/pda_2002_6_development-of-the-antimicrobial-effectiveness-test-as-usp-chapter-51.pdf

A3: To add to this, I seem to remember some long-lost late-night discussions with Scott about AET microorganisms. If my foggy memories serve me (which is in doubt), I seem to recall that one of the reasons for those ATCC choices may have been that they did not have any specific resistance to antimicrobials. Other than that, Scott's article, as Phil mentions, contains a lot of good history on the test. Or they simply put up a dart board with the microorganisms on it and saw what came up. Your guess is probably as good as mine lol.

A4: I go with the dart method.

QC of Media Previously Accepted Lot

In the current <1117> effective in August, there is a clarification to the statement for QC of media:

For qualitative and quantitative comparison, direct physical comparison with a previously tested batch is not necessary.

This is a classic micro discussion and topic of many opinions.
I would like some input on how companies are performing this comparison when testing growth media for QC growth promotion qualities. What is the industry expectation and have any 483s been issued regarding the QC of media testing?

A1: I took this to mean you can compare the current GPT results to past data without directly setting up concurrent GPT with batches.plates from the different batches.

When I directed a lab I maintained a control chart and added the new lot data when it was obtained.

A2: If you are comparing the current GPT to past data, what did you use as acceptance criteria? I took this to mean that if you are performing a comparison of numerical results, you should still be considering a factor of 2 (50 -200%) as a guide. And it looks to me that despite this clarification in <1117> , Chapter <61> still seems to require a direct comparison of the incoming lot and a previously approved lot of media.

A3: I read it this way as well...no need for a concurrent GPT confirmation on an old lot...just compare numbers.

A4: Control charting rules, i.e., count within 2SD with no adverse trends.

The previous lot often is not available or past expiry. Using control charting leverages the past history of GPT results.

A5: Do you consider potential variability in inoculum levels a concern?

A6: There is always variability in the inoculum so it factored in control charting.

A7: We have variability in inoculum all the time. Usually, when using pre-made kits or using our own suspensions, the acceptable range is "not more than 100 CFU". The usual aim is 10-100, and the variability in different test sessions and between lots varies. We wouldn't want to fail a lot because one week we had counts around 20 and one week they were around 80. (less than the 50%/over the 200%). How do you suggest avoiding this?

A8: Are you using Quanticults [name redacted]? I've experienced this before with qualnticults and found that warming the vials (with the desiccated organism on the cap) in an incubator, and ensuring the suspension is thoroughly vortexed helps. Other than that, check accuracy / calibration of pipettes.

A9: I think that [name redacted] point is that Quanticult does not claim to have a consistent level of organism per vial. They will only guarantee that each vial contains <100 CFU. So, her counts of 20 one week and 80 the next week are likely to be accurate.

A10: (Yes, we use some quanticults and follow strict preparation instructions. We also have pipette checks/calibrations often.)
This variability will be seen with quanti-cults as well as home-prepared suspensions. Currently we are using up twice as many supplies and time since we are performing double GPQ (current lot against previous lot concurrently for every shipment received or lot made on site).
We can save a lot of time and money not doing it this way but have been unable to overcome the topic of discussion - variability when dealing with such small counts - especially when we are not using the same environmental organism that was used two months ago for the last shipment of media (environmental rotation requirement). Suspensions die, new lots of commercially-prepared organisms come in, etc.
I don't see the value added in performing a quantitative comparison when working with 100 CFU or less. I also don't know how to get around the requirement and remain compliant.

A11: In my experience vials of quanticult contained 1000 CFU (with a +/- range) and I'd be able to inoculate 10 plates with one vial, and get consistent results.

That said, <62> does have that requirement to compare to the previous lot. Until that's deemed irrelevant and removed. In theory I don't think it's completely irrelevant. Of all the things I've heard on this discussion, I think keeping a simple running control chart (+/- 2 standard deviations) of every lot / shipment is the best way of not only comparing to the previous lot but monitoring for any anomalies over time - whether they be due to supplier issues or the execution of the test.

Another idea is trying cryobeads if you're finding the variability of quanticults, etc, to be too high. There's a bit less variability using a freeze dried ball of 100 CFU than ensuring a quanticult is sufficiently homogenized while at the same time minimizing risk of damaging the organisms with over-vortexing. Then again I don't think cryobeads are necessarily inexpensive.

Growth Promotion testing

I am curious as to the expectations for growth promotion testing of incoming growth media. I have always followed USP <61> which requires "*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." *I have always used this criterion for all growth media. I have noted that USP <1117> Microbiological Best Laboratory Practices, seems to contradict the requirement in <61> - "For qualitative and quantitative comparison, direct physical comparison with a previously tested batch is not necessary."

Does the requirement to test a new batch of media against a previously approved batch only pertain to Chapter <61>? Would it be acceptable to use a non quantitative method such as streaking the plates for media not used for bioburden USP <61> testing?

USP Probiotic biological assay procedure

Does anyone have knowledge about probiotic microbial assay? Please share the procedure for probiotic assay.

A1: I assume that you are looking for an enumeration test method for determining the number of probiotic organisms that are present in a product formulation. However, I'm not aware of an official test method for enumerating probiotic organisms in a product formulation, but it is my understanding that there is no single test method that can be used to detect all of the different types of probiotic organisms that are being used today in probiotic products. It is common to see a mixture of different probiotic organisms in a single product formulation.

For enumerating Bifidobacterium and Lactobacillus species in a product formulation, most people will use the pour plate method that is indicated in Chapter 3- Aerobic Plate Count of the FDA BAM. Instead of using Plate Count Agar that is present in this chapter, they will use either MRS Agar or APT Agar for enumerating the number of probiotic organisms in the product formulation.

If you are interested in obtaining separate Lactobacillus and Bifidobacterium counts in a single product formulation, I have also seen people use Columbia agar base media supplemented with lithium chloride and sodium propionate and MRS medium supplemented with neomycin, paromomycin, nalidixic acid and lithium chloride for the selective enumeration of bifidobacteria in dairy products. In addition, Bifidobacterium Selective Media (BSM) can be used to detect the number of Bifidobacterium species in a product because it will inhibit the growth of Lactobacillus and Streptococcus that are also being used in probiotic formulations.

If both Lactobacillus and Bifidobacterium species are being used in a product formulation, the number of Lactobacillus can be determined by subtracting this number of Bifidobacterium count from the total count obtained by using either MRS or APT Agar

In addition, there is a procedure in ISO22981:2010 that describes an enumeration of presumptive Bifidobacteria test method for dairy products by using the TOS-MUP Agar. TOS-MUP Agar is a highly selective microbial growth agar for detecting Bifidobacterium by suppressing the growth of Lactobacillus.

I have attached some references for you to read.

References:

Davis, C. Enumeration of probiotic strains: Review of culture-dependent and alternative techniques to quantify viable bacteria. Journal of Microbiological Methods. Volume 103, 2014, Pages 9-17.

Roy D. Media for the isolation and enumeration of bifidobacteria in dairy products. Int J Food Microbiol. 2001 Sep 28;69(3):167-82. doi: 10.1016/s0168-1605(01)00496-2. PMID: 11603854.

A2: I have to test one sample in which I have to perform 'detection of L.acidophillus' so I need procedure for that.
Do you have? It should be comply USP.

A3: Please reference USP Chapter <64> Probiotic Tests. It covers enumeration, testing for contaminants and strain typing specific to Lactobacillus. and Bifidobacterium.

Note that there are also active probiotic monographs available in the USP including L. acidophilus.

A4: As [name redacted] said for FDA BAM, it also has good information.

A5: My experience has been with probiotic products containing both Lactobacillus and Bifidobacterium species. If you are only doing lactobacilli, it is my understanding that USP Chapter 64 does offers a method in which the sample is dissolved in MRS broth, homogenised with a blender or stomacher, pre-incubated at room temperature, re-homogenised, then diluted 10-fold in a Peptone diluent, and MRS Agar is used as the recovery agar. The method presented in USP Chapter 64 is only for determining the number of lactobacilli in a product and not for the other types of bacterial species that can also be used in a probiotic product. In addition, I do question as to why the homogenized sample is pre-incubated at room temperature in this USP method for determining the lactobacilli count.

A6: Please guide for assay calculation of three different plates. As i have taken 1ml of aliquot from 10*-3 , 1ml from 10*-5 and 1ml from 10*-7. I got TNTC for 10*-3 and 10*-5. so how is it considered? because i have taken sum calculation of plate 1, plate 2 and plate 3.

A7: In general, most probiotic products will contain at least 1,000,000 CFU/gram. Due to probiotic organisms die-out during its product shelf-life, a 10% overcharge for the probiotic organism is used at the time of manufacturing to ensure that the label claim of 1,000,000 CFU is maintained during its shelf-life.

To perform a plate count of a probiotic product, 1:10 serial-dilutions are performed from which 2 x 1.0 ml-aliquots for each serial dilution are plated. For a product with 1,000,000 CFU/gram, duplicated 1.0-ml aliquots are plated from the 10-5, 10-6, and 10-7 dilutions to obtain a count. I have never seen someone try to obtain a count of a probiotic product with the dilutions that you had used in which you are trying to use a sum for each of the dilutions.

<1229.5> Biological Indicators for Sterilization

We are using the Biological Indicators vials for Ethylene Oxide Sterilization. My main concern is as below:

In USP39 - Chapter <1035> Biological Indicators for Sterilization having the characteristics for commercially supplied BI systems, which shows the below mentioned limits for BI vials;
1. Range of D-values: 2.5 to 5.8 minutes
2. Survival Time: 10 to 27 minutes
3. Kill Time: 25 to 68 minutes

But in USP42 the same limits are removed. So kindly guide me from where i can get these limits for BI vials?

A1: The d-values, organism(s), titers, etc. have been moved into the applicable ISO 11138 series documents based on the associated sterilant.

ENVIRONMENTAL MONITORING SETTLE PLATE STANDS

I have recently got to learn about petriplate stands that can be utilised during environmental monitoring using the settle plate method. Does anyone know what the recommended height of these settle plate stands should be and any design recommendations for the stands, i.e, i have been reading about these stands coming either as horizontally surfaced stands or inclined/ slanted stands. How would these two factors possibly affect the quality of the settle plate method during environmental monitoring?

A1: I have seen stands at an angle but I'm not sure where or why that originated. I don't know of any document that states the settling plate should be at an angle. That being said, the plate should be at working height, capturing particles out of the air where they may fall into the product or surface.

A2: Earlier in my career at the NY Blood Center we used retort stands which hold the Petri plates and standardized their location.

That reflected my organic chemistry training as an undergrad.

A3: Settle plates should ideally be positioned such that laminar flow is hitting it directly. I've never seen them not pointing straight up (I.e. no angle..). Positioning could be determined and justified via recorded smoke studies.

A4: The angled plate was the result of a study by Upjohn in the mid 80s, where they showed the air flow pattern over a plate on a flat surface and an angled surface in a unidirectional airflow area was better on the plate on an angled surface. I am still looking to see if I have the paper in my files.

Regarding MLT

Respected Dear Fellows, I need some guidance on the MLT Maximum Acceptable Criterion for Non-Sterile Products.

In MLT general chapter of all Pharmacopoeia, following statement has written:

When an Acceptable Criterion for Microbiological Quality is prescribed, it is interpreted as follows:

10e1: Maximum Acceptable count=20
10e2: 200 and so on...

Some experts said that the limit for Non-Sterile Products is
10e1=20, 10e2=200, 10e3=2000 and so on, but in general maths rule 10e1=10, 10e2=100, and so on...

Now what is the need or logic behind above mentioned statement of all Pharmacopoeia???

Why the limit of Non-Sterile Products has mentioned as 10e1=20 instead of 10e1=10??? And also why the limit of products not mention in numbers like NMT 1000 cfu or 2000 cfu instead of exponential notation like 10e1, 10e2 and so on???

Can someone guide me in this regard???

Microbial Enumeration Specifications for Purified Water and Water For Injection

In the past, USP had microbial count specifications present in the USP monographs for Purified Water and Water For Injection. However, these microbial count specifications have been removed from each of the monographs. It is my understanding that the microbial requirements for Purified Water and Water For Injection had been removed because they would a cause unnecessary burden for some users with meaningless and/or inconsequential or inappropriate requirements, e.g., water used for many laboratory analysis.

In Section 9.4.4 of USP Chapter 1231 – Water for Pharmaceutical Purposes, it is stated that users should establish their own quantitative microbial test specification suited to their water uses. But these values should not be greater than 100 CFU/ml for Purified Water and 10 CFU/100 ml for Water For Injection unless specifically justified, because these values generally represent the highest microbial levels for pharmaceutical water that are still suitable for manufacturing use.

From my perspective, I do not see how someone would be able to justify the usage of higher enumeration levels for Purified Water and Water For Injection.

My questions to the followers of this forum are as follows:

Are people using these values in Chapter 1231 of not greater than 100 CFU/ml for Purified Water and 10 CFU/100 ml for Water For Injection as their quantitative microbial enumeration specification or are they establishing more stringent microbial enumeration levels for their own Purified Water and Water For Injection samples?
Has any regulatory authorities conducting cGMP inspections of manufacturing facilities have questioned if you are using the enumeration limits in USP Chapter 1231 instead of establishing their own microbial enumeration specifications for Purified Water and Water For Injection?

If microbial test data is showing that low levels (e.g., less than 10 CFU for 100 ml for Purified Water from ozonated and hot water circulating systems) are being obtained for Purified Water samples, how are companies explaining why they are not using more stringent enumeration levels to a regulatory authority when the test data justifies the usage of a lower enumeration level?

A1: Based on our many years of work with water systems, I can offer the following:

Most companies are using the USP 1231 recommended action levels.
I have experienced some regulators question why the limits are not lower, based on historical data. But this has not resulted in any 483 observations, since USP 1231 levels have historically been accepted. But see my next point.
Many companies I have worked with do set alert limits or even action limits lower than the USP 1231 recommended levels, based on historical data. In some cases, the USP level is a “specification” such as 100 cfu/mL for purified water, with a lower level for an action limit (e.g., 50 cfu/mL) and even lower for an alert level (based on the capability of the system).

A2: I'll share my thoughts to your questions with our current practices with our purified water systems.

1. We use the three-tier approach described in USP <1231> Section 9.4, meaning we have alert level, action level, and specification. Although the USP purified water monograph only offers reference to USP <1231> for microbes, the Ph.Eur. *bulk* purified water monograph has that same 100 CFU/mL hard limit for action level. Furthermore, the Ph.Eur. monograph for purified water in *containers* presents the micro contamination limit as a true specification at 10^2 CFU/mL. We do market products in Europe so we need our water to comply with Ph.Eur. requirements. We've adopted the 100 CFU/mL limit for bulk purified water as a specification since the previously mentioned compendial references indicate that's a point where the microbiological quality of water becomes questionable. That leaves the alert and action levels. For us, both alert and action control levels are based on the historical performance after analyzing the data.

2. After installation of each of our water systems, we've had regulatory inspectors go over the qualification, validation, and monitoring procedures in very intense water-focused audits and there were no issues with our three-tier approach above.

3. I think the key is to determine at what point is a statistically determined limit based on historical data not value-added for process control and justify a cut-off limit through a formal risk assessment. If you sample and test 1000 water samples and they are all zero, but then sample 1001 has single CFU recovered it will be flagged as an unusual event statistically. Is it value-added to investigate? The same compendial guidance says a higher grade of water, WFI, can have counts up to 10 CFU/100mL. To me, it doesn't make sense to hold a lower grade of water to more stringent levels than what is allowed for a higher grade of water. An important point that needs to be made- if you are seeing a lot of zeros you may want to evaluate your samples size. For example, if you only test 1mL of water and always see zeros you can get into trouble with inspectors. *Read footnote "b" under Table 3 in USP <1231>: Sample size must be appropriate for the expected microbial count of the water in order to derive statistically valid colony counts.*

A3: In my experience working in biologic DS manufacturing sites that the PW and WFI microbial count action levels were aligned with <1231>. I have not been questioned from an inspector to justify those levels. The last question is a good one and thankfully I haven't gotten it in an inspection.

A4: We've dealt with this same issue with multiple clients and have had regulatory observations. My comments below seem to reflect the most common observations I've had with USP Purified Water (both current practice and what the agency is looking at).

1. Most non-sterile companies we work with initially use the <100 cfu/1mL specification and start off testing using a 1mL pour plate (in duplicate) with 2-3 day incubation at 30-35C. We ALWAYS recommend performing a media qualification study as recommended in USP <1231>, but of course, that isn't cheap so they follow the purified water "regulatory requirements". This reference in USP has been used by both regulatory inspections and by us to help push companies toward a proper heterotrophic plate count (HPC) process:

"Section 8.5 Every water system has a unique microbiome. It is the user's responsibility to perform method validation studies to demonstrate the suitability of the chosen test media and incubation conditions for bioburden recovery. In general, users should select the method that recovers the highest planktonic microbial counts in the shortest time, thus allowing for timely investigations and remediation. Such studies are usually performed before or during system validation."

2. We recommend that companies start with using the <100 cfu/1mL spec and then re-visit the specifications annually. We suggest lowering your specs depending on your test results. We also recommend that the companies test using 1mL AND 100 mL. The 100mL volume allows you to see potential problems (with the growth of biofilm) before they become action items. We have seen regulatory bodies question the enumeration levels, especially if they have been getting <1 cfu/1mL as far as the eye can see. At that point, the questions seem to be about whether the test methodology is correct (see my comment above). We have also seen regulatory comments regarding the ability of media to recover certain types of microorganisms (with Bcc being the pre-eminent request). We have performed growth promotion testing using additional microorganisms to show the ability of the media to recover potentially objectionable microorganisms, The regulatory bodies have also been requesting testing of water systems using a "validated" method, and the go-to testing methodology has been USP <60>.

My 2 cents worth of this discussion. I'm sure there will be plenty of additional comments.

A5: I think that, in general, many facilities set their limits based upon what the system is able to do. For example, on company has a specification of <1 cfu/100 mL since they have a very tightly controlled system. If you set the limits too high, even 10 cfu/100 mL, then you may never detect that you have a problem. Hence, you may end up with a contamination even further on in the process and you won't have the information available to help with the investigation. I know of an instance in which a WFI system routinely had low counts, but since they were below the 10cfu limit, the firm didn't even perform ID's.

A6: I agree with [name redacted] 3rd point, when you have lots of zeros then you have to increase your sample.

But, I want to ask a question to Robert about following lines:
The Ph.Eur. monograph for purified water in *containers* presents the micro contamination limit as a true specification at 10^2 CFU/mL.

So, in above statement, what would be the limit of CFU/mL, either 100 CFU/mL or 200 CFU/mL???

Because, so many intellectuals, take the meaning from 10e2 of 200 instead of 100, and also in Pharmacopoeia stated that line = 10e1: maximum allowable count = 20, 10e2: maximum allowable count = 200, 10e3: maximum allowable count = 2,000...

Please clear it.

A7: Ali the 10e1: maximum allowable count = 20, etc. refers to monographs for bioburden analyses (e.g., USP 61) and not necessarily true for an action level for water analyses. So, I would be conservative and interpret a specification at 10^2 CFU/mL as not more than 100 cfu/mL.

A8: As a consultant, I have seen many times that companies will not perform identifications of water borne isolates from their water test samples unless the level was above their alert level. I'm very uncomfortable with this practice and I do not agree with it. I always want to know the identifications of the water isolates that are being obtained on a routine basis to see if I'm going to have the possible development of a potential issue. I will admit that maybe that I'm being too conservative in having water borne isolates identified to the genus/species level no matter what is the count.

A9: I think any attempt at saving money and guessing ID's is a mistake. I'd rather know the ID and start my investigation WAY before I hit my action level. Have a microbiologist get out and walk the plant...see if there are any opportunities to stop or mitigate an action level contamination event.

A10: But a question arise here, then why in pharmacopoeia regarding MLT (USP 61) this statement has shown, why not the exact number mention like NMT 1,000 or 2,000, why has shown this 10e1: maximum allowable count = 20???
Because when microbiologists or even other than microbiologists go through the table of allowable limits (e.g. USP 1111) they consider 10e2 = 100 & 10e3 = 1,000, and this statement 10e1: maximum allowable count = 20, make them confuse...

Kindly guide in this confusing statement.

A11: Yes, it is confusing. This was added to USP 61 to account for variability on plate counting But this ONLY applies to USP 61.

A12: Right, but for MLT almost every lab perform in duplicate and even some perform in triplicate and get the average result to show in the reports, so, the variability matter is finished there...

As far as my understanding concern, the limit of USP 61 is NMT 1,000 CFU but when any sample cross the limit but it not cross the maximum allowable count of 2,000 CFU, then the sample will be allowed to release... And this is the understanding which I got...

What other colleagues think about it, am I right in this context???

A13: These are the types of topics that make microbiology as interesting as it is.

The real question is not the limit but the source and your reaction.

If you want to apply a 200cfu/ml limit then, as a microbiologist are you comfortable with that?

If you set that limit and get a 100-200cfu/ml result are you going to ignore that?

Do you understand and appreciate those results when they occur?

Counts of this nature are likely to be sourced by a fundamental problem with your testing and in-use procedures or, your system and its cleaning procedures are incapable of removing high bioburden.

What you need to be concerned about is which is the source.

So, I'm curious to see definitive answers like Michaels but advise you not to lose sight of your actually task of understanding and applying risk mitigation.

A14: This topic also came to the fore in Joel Russo's contemporary PMF thread, "Question regarding limits in <61>" so apologies to all for the duplication but here it is again.

Like many of my colleagues, I have for years just accepted that it was a "for information only" type element , providing a theoretical estimate for what a defined specification level might mean theoretically in terms of actual result range.
But apparently its finding use as a means of introducing tolerance into the actual specification in actual release testing circumstances.
Hence, my interest in digging down into the topic and searching for consensus.
So for those who have accesses the same contribution in Joel Russo's thread apologies but here it goes again.

Regarding USP<61> “Interpreting the Interpretation”

This is also a discussion subject in a contemporary PMF Forum thread, Microbial Enumeration Specifications for Purified Water and Water For Injection.

” Interpretation of the Results” USP<61>

e.g., 102cfu: maximum acceptable count = 200

As has been mentioned by others in previous and a contemporary thread, this relates to allowing the long used theoretical estimate of 50 – 200% variability for traditional micro testing methods, be included in the interpretation of a what a defined specification means in terms of result range. (at least as far as I can see).

So, for a plate count of 100 fu recorded for a test aliquot, the actual count for such test aliquot could be anywhere between 50 - 200cfu (in theory).

The key words for me, in this rather vague and confusing section is “interpretation”.

I have always assumed it to be a theoretical consideration and that it’s not actually providing any more tolerance / width to the specification, but just reminding the user that when a test meets the defined specification of < 100cfu, the test result could realistically be up to 200% higher.

That noted the actual specification is still <100cfu , e.g., 110cfu would be an OOS.

One might ask, then (at least I have) what was the intent of including this extra information?

The only answer I could arrive at eventually, was that it reinforces the fact that high levels of inherent variability in Microbiological tests should be considered when establishing test limits / levels and specifications.

In other words, given this variability, actual release specifications might need to be tighter than that provided for in the regulations when such methods are used.

As an example, where possible if tasked with meeting a client specification of <100cfu, given the defined variability, I have always strived to test to a <50cfu action level (action limit) or even better an internal specification of < 50cfu (where feasible from the point of view lab testing efficiency) in order to have good confidence that I have a statistically sound and defensible test program that won’t get immediately shredded in the event of future complaints or issues.

The stance was that at least if my interpretation of the section is wrong, I am erring on the side of “safety" and promoting good quality, not just compliance.

To those who interpret this section of USP to mean that given a <100cfu specification, one could actually release with actual test results up to 200cfu, if that were the case, my question is would not one have to

assume that a result for a sample yielding an acceptable count of 200cfu could theoretically translate to 400cfu,?

Does that make sense. Personally, I would not accept material on a <100cfu specification if there was the remotest chance that it could actually contain 4x defined limit.

What are customers asking for or what do the think they are getting when they ask the provider to meet a USP monograph specification set at <100cfu?

e.g., the USP states < 100cfu and that's what the client wants irrespective of what such specification might mean in terms of theoretical microbial content (variability of MLTs might not be their thing).

I have been on the client side as well as the supplier end, and have had occasion to reject vendor supply material with counts of > 110cfu against an acceptance specification of < 100 and with all due respect, if a supplier were to try to justify acceptance on the 50 - 200% variability issue, I would also remind them that they were already allowed tolerance to provide material in the 50 - 100cfu range, material that could theoretically be outside the specification if one were to use the same justification.

Finally, given that the USP is not the client, can one assume that the USP introduced the interpretation just to provide a little information on inherent test variability to the tester and their intent was not to change the specification "goal posts" - so to speak.

It's been so long out there and unamended maybe it was not considered such a big deal at the time and has just grown wings as we have become ever more focused on Microbiology Quality.

Even reading the USP 61 section now, it's not clear to me what actual intent was. I really wonder why the USP has not at least clarified their intent with this section as it has been a common topic of confusion for as long time.

All feedback is truly appreciated, as I have arrived at my interpretation over time, but never had it confirmed or rejected, but it would be good to finally put this to bed, particularly if I'm wrong.

Anyone, have the ear of the USP?

A15: I cannot understand why this issue comes up repeatedly. The concept of maximum acceptable limit is clearly stated in both USP <61> and <1111> , which are chapters harmonized with the Ph. Eur. and the JP so the concept has global regulatory acceptance. The concept overcomes the absurdity of passing a product with a microbial count of 100 cfu/g and failing it with 101 cfu/g and acknowledges the low precision of microbial counts, i.e., 15 to 35% RSD at a 100 cfu/g level. The USP expresses the microbiological requirement as not more that 10^2 cfg/g with the maximum acceptable count 200 cfg/g.

A16: 100% agree with your points and your expressed points are those which I also tell or express to my colleagues who are not microbiologists but the fact is that those person who are working in Microbiology field are not getting the actual meaning and not keen to search what the guidelines are saying to us and also what guidelines are wanting from us...

A17: I don't think that you are conservative. Water quality is too crucial to many products. In addition, ID's of what is in the water can give you an early warning of a developing problem. Since most well engineered systems these days have few to no recoveries in routine operation the requirement for ID's should not be onerous, but can be extremely helpful.

A18: Chapter 61 and 71 growth promotion specifies the following: For solid media, the growth obtained should not differ by a factor greater than 2 from the value calculated for a standardized inoculum.

In this sentence we will differentiate three basic aspects: the mathematical, the semantic and we will end with the microbiological.

Mathematically, a factor means multiplying by a value, in this case the one suggested by the monograph to form a product.

Semantically Differ in Latin Differre means to lead in different directions or to be different in the case that we will study we could analyze it as a range.

Microbiologically, the growth behavior of microorganisms together with the standardized microbial load is not an exact process but rather presents variables that, being controlled in different aspects, give us a mere idea of the amount of inoculum.

That said, when we inoculate a small number of microorganisms (no more than 100 cfu) we would expect a count between 1 and 100 cfu or maybe 70% according to company policies, but this does not always happen, that is where the interpretation and reasoning they play a fundamental role.

In this case, the inoculum is 100 cfu and the recovery can differ in factor 2, that is, between 50 cfu and 200 cfu, so if you recovered 180 cfu there would be no problem, the inoculum was correct and there was no error in it, if for On the contrary, your recovery was less than 50, the possible variables that influenced it should be verified, in the same way if it was greater than 200.

In any case, this does not apply to the water limits, since the microbiological matrix is not known and cannot.

Presence of Enterococcus faecium

I have identified Enterococcus faecium in WHEY PROTEIN supplement, This organism was identified by Vitek2 with 3 times retesting and same results have occurred. The organism is mentioned as one of objectional organisms in the and BAD bug foodborne organism-FDA. However, the presence of enterococci(as a probiotic) in dairy products is a matter of debate as i understand from my research on the internet.

Anybody have experience in this matter , is the presence of Enterococcus faecium is allowed in whey protein powders used in bodybuilding?

A1: Enterococcus faecium is expected to be found in dairy products and other processed food. As prominent gram positive coccus from the human intestine they are often used as indicators of fecal pollution and are viewed as more reliable than coliforms.

The Vitek 2 Gram positive identification card should be reliable for its identification

However, the bacterium is often responsible for urinary tract infection and may be viewed as an opportunistic pathogen. These infections probably arise from the patient's own microflora.

A2: Enterococcus faecium is commonly found in the gastrointestinal tract of humans and animals. It is considered to be a pathogen because it can cause urinary tract infections, bacteremia and endocarditis in humans. It is common to find Enterococcus faecium in foods as a contaminant. Enterococcus faecium is a common isolate that can be found in raw milk. Enterococci can survive pasteurization of raw milk so that it is not unexpected that Enterococcus faecium would not also be present in whey. You are correct that there is no consensus on the acceptance of the presence of Enterococci in foodstuffs. There is even evidence that nosocomial strains of Enterococcus are genotypically different from strains that are found in gastrointestinal tracts.

What levels of Enterococcus faecium are being detected in the whey powder? Has Enterococcus faecium been found in additional batches of the whey product? For a pharmaceutical or cosmetic product, I would

consider the presence of Enterococcus faecium to be objectionable no matter what is the level. For a dietary supplement that is made from milk, I'm not so sure. If it was at high levels (e.g., greater than 100 CFU/gram) in the whey product, I would definitely reject it. If it had not been found in additional batches of tested whey products, I would also reject the current batch. If Enterococcus faecium is at low levels (e.g., less than 10 CFU/gram), you need to perform a risk assessment to determine if low levels is acceptable or not acceptable to have in a whey product that is made from milk.

Sometimes in Microbiology that there is no clear cut answer to a question to an issue that you are having.

A3: Hence the need to perform a risk assessment on whether E. faecium, at any level, is acceptable.

A4: Can Enterococcus faecium grow anaerobically? Is there a possibility of isolating this organism along with Clostridium diff? I was shocked to find E. c in my ATCC culture of C. diff growing anaerobically!!! It was very strange!!! We don't handle any faecal matters in my lab! C. diff was used to test disinfectants. So I was wondering what is the possibility of this appearing as a contaminant?

A5: It is a facultative anaerobe, so yes it can.

A6: As previously mentioned by Don & Tony, naturally occurring wild type strains of Enterococci are part of the normal gut flora of many animals including cattle and like most bacteria (both common and exotic) can act as opportunistic pathogens under the right circumstances.

The fact that they are generally more resistant to heat than Grams Negative Bacilli (GNBs) and common commensals of the mammalian GI tract makes them useful indicators of poor sanitation / faecal contamination etc. This attribute is possibly most relevant in this case.

Regarding presence in whey, unless the whey is a by-product of a "Raw" milk cheese production process, it will likely have been heat treated during the milk pasteurisation process. Depending on the given process it may also have been subjected to a further heat treatment or pasteurisation step.

Much depends on the defined heat treatment process applied and though Enterococci are s generally higher on the spectrum than GNBs with regard to heat resistance, a standard Batch or UHT Pasteurisation process should be well able to significantly reduce Enterococcal contamination to the required level (normally negative by test). I am also assuming Bioburden levels are within validated or normally established limits for the process. Thus, I would not normally expect to see Enterococci at detectable levels in a pasteurised foodstuff using a standard test method & standard sample quantity (1ml of 1:10 dilution).
With respect to Probiotic formulations using Enterococci, the commercial use of probiotics requires that the actual strains used, are well characterised (phenotypically, biochemically and genotypically) to ensure low virulence, low antimicrobial resistance patterns and genetic stability. Such cultures are prepared and maintained under controlled and approved conditions and generally have an established label claim. It's not clear from your post, but as far as I can tell your product is not a probiotic and therefore the potential probiotic properties of specific strains of the organism would be irrelevant to your context.

Do you routinely test the product for Enterococci.? If not, what initiated this action/ investigation, an OOT (Out of Trend) or OOS (Out of Spec) etc.?

I am making an assumption that your testing of the product and subsequent identification of Enterococcus faecium was the result of an investigation into atypical Bioburden / TAMC levels or an excursion in product of same – though I might be wrong, and it might be that you do employ a routine release spec for Enterococci with your whey product? As Don mentioned, not knowing the relative level of Enterococcal contamination, I can only surmise the test scenario, but from this distance and apparent context,, I'm going with my gut (commensal enterococci and all) , and would recommend rejection of the product, considering the contaminant is often indicative of sub-optimal processing, poor sanitary practice, or equipment cross contamination as also inferred by the position taken by the FDA Bad Bug book.

A7: It is my understanding the E. coli can grow both aerobically and anaerobically. It is also my understanding the Enterococcus faecium is a facultative anaerobe which can grow either in the absence or presence of oxygen.

If you are doing disinfectant testing with C diff and you obtained an E. coli, I would say that it is possible that cross contamination has occurred somewhere.

A8: It is Enterococcus faecalis is what was recovered under anaerobic condition, not E.coli. That's what puzzles me. I had it sent out to accugenics for identification.

A9: Although Vitel 2 should be able to ID E. faecium correctly, i would recommend to get the verification using genotypic identification. It may help with the investigation.

Question regarding limits in <61>

I have a question regarding the section in <61> that specifies:

<< When an acceptance criterion for microbiological quality is prescribed, it is interpreted as follows:

10^1 cfu: maximum acceptable count = 20;

10^2 cfu: maximum acceptable count = 200;

10^3 cfu: maximum acceptable count = 2000;

and so forth. >>

With regard to the above, if an internal spec is set to 100 (TYMC) and the result is 120, is this product meeting GMP requirements?

Another more general question is what is the driving factor for this prescription in <61>? I'm a bit puzzled by the rationale behind this.

I've spoken to experts in the industry that indicated that as far as FDA is concerned, the internal spec will apply, regardless of this prescription. Has anyone had experience with FDA accepting the USP prescribed limit over internal set limits that are different (set as half, specifically)?

A1: As well as all those discussing Point No. 3, USP<61> Interpretation the Specification, in the corresponding PMF Forum thread, Microbial Enumeration Specifications for Purified Water and Water For Injection.

Lots of specification threads seem to be converging, so I'm giving my "tuppence hapenny's" worth and asking for clarification in relation to the old chestnut in USP<61>. Maybe someone has dug down on this before and I missed it.
There is probably very little different or new in my contribution and maybe if lucky it has value for those relatively new to the world of Microbiological Quality.
If any of it stimulates discussion well and good,

1: Release Specification
Re. FDA rejection of material outside internal specification:
I Would be amazed if it was otherwise.
If the formal Specification is <100cfu, a result of 120cfu is an Out of Specification (OOS) result, irrespective of context, or any other interpretation, official or otherwise.
Once documented & approved within the QMS, a specification (whether internal / external) becomes the defined formal instrument with which one determines the suitability of a given material /product for its

intended purpose or market etc. Being unsuitable, inappropriate or downright incorrect, means it needs to be revised asap or invalidated (issue a deviation, NCR etc and all product / material gets quarantined, and disposition further adjudicated), but as long as a specification is current/valid, it is the effective requirement and there is no playing fast or lose with its application.
Any different interpretation needs to be integrated into a formal revision through the approved Change Control process.

2 “is this product meeting GMP requirements”?
I'm probably splitting hairs, but it is useful in my opinion when reviewing adverse quality events to uncouple GMP compliance issues from Product Quality (Microbial Quality) Issues, though often one will lead to the other.
Personally, I don’t think of a material / product as having to meet GMP requirements.
Suitability or appropriateness of the specification is not directly related to GMP and so the product per se is not required to meet GMP requirements.
In the example above (1), the material result is an OOS result thus requiring a Failure Investigation to determine whether the material is quality compliant i.e. Conforms to the specification or not. Unless the result was determined to be a Laboratory Error during the OOS Investigation and as such invalidated, the material is considered Non-Conforming and rejected.

In the event that the Failure Investigation (OOS investigation / associated CAPA review) is unable to identify system excursions i.e. the system is in a normal state of control, as established (when validated), and so the process is still considered GMP compliant even if the product is rejected. That’s where the suitability or appropriateness of the spec would come into play.

3 - Regarding USP<61> “Interpreting the Interpretation”
This is also a discussion subject in a contemporary PMF Forum thread, Microbial Enumeration Specifications for Purified Water and Water For Injection.
” Interpretation of the Results” USP<61>
e.g., 102cfu: maximum acceptable count = 200

As has been mentioned by others in previous and a contemporary thread, this relates to allowing the long used theoretical estimate of 50 – 200% variability for traditional micro testing methods, be included in the interpretation of a what a defined specification means in terms of result range. (at least as far as I can see).
So, for a plate count of 100 fu recorded for a test aliquot, the actual count for such test aliquot could be anywhere between 50 - 200cfu (in theory).

The key words for me, in this rather vague and confusing section is “interpretation”.
I have always assumed it to be a theoretical consideration and that it’s not actually providing any more tolerance / width to the specification, but just reminding the user that when a test meets the defined specification of < 100cfu, the test result could realistically be up to 200% higher.
That noted the actual specification is still <100cfu , e.g., 110cfu would be an OOS.

One might ask, then (at least I have) what was the intent of including this extra information?
The only answer I could arrive at eventually, was that it reinforces the fact that high levels of inherent variability in Microbiological tests should be considered when establishing test limits / levels and specifications.
In other words, given this variability, actual release specifications might need to be tighter than that provided for in the regulations when such methods are used.
As an example, where possible if tasked with meeting a client specification of <100cfu, given the defined variability, I have always strived to test to a <50cfu action level (action limit) or even better an internal specification of < 50cfu (where feasible from the point of view lab testing efficiency) in order to have good confidence that I have a statistically sound and defensible test program that won’t get immediately shredded in the event of future complaints or issues.
The stance was that at least if my interpretation of the section is wrong, I am erring on the side of “safety" and promoting good quality, not just compliance.

To those who interpret this section of USP to mean that given a <100cfu specification, one could actually release with actual test results up to 200cfu, if that were the case, my question is would not one have to assume that a result for a sample yielding an acceptable count of 200cfu could theoretically translate to 400cfu,?

Does that make sense. Personally, I would not accept material on a <100cfu specification if there was the remotest chance that it could actually contain 4x defined limit.

What are customers asking for or what do the think they are getting when they ask the provider to meet a USP monograph specification set at <100cfu?
e.g., the USP states < 100cfu and that's what the client wants irrespective of what such specification might mean in terms of theoretical microbial content (variability of MLTs might not be their thing).

I have been on the client side as well as the supplier end, and have had occasion to reject vendor supply material with counts of > 110cfu against an acceptance specification of < 100 and with all due respect, if a supplier were to try to justify acceptance on the 50 - 200% variability issue, I would also remind them that they were already allowed tolerance to provide material in the 50 - 100cfu range, material that could theoretically be outside the specification if one were to use the same justification.

Finally, given that the USP is not the client, can one assume that the USP introduced the interpretation just to provide a little information on inherent test variability to the tester and their intent was not to change the specification "goal posts" - so to speak.

It's been so long out there and unamended maybe it was not considered such a big deal at the time and has just grown wings as we have become ever more focused on Microbiology Quality.

Even reading the USP 61 section now, it's not clear to me what actual intent was. I really wonder why the USP has not at least clarified their intent with this section as it has been a common topic of confusion for as long time.

All feedback is truly appreciated, as I have arrived at my interpretation over time, but never had it confirmed or rejected, but it would be good to finally put this to bed, particularly if I'm wrong. Anyone, have the ear of the USP?

A2: I saw a client using the "200" limit when it suited their results over a period of three full years, but noticed that when it made no difference, cited their "100" cfu limit. That all changed at one point in time, where thereafter they held themselves to the internally set "100" limit, consistently. That timeframe lined up with FDA inspection and after speaking with the lab, confirmed that's indeed why.

That's all I need to know. And agreed, microbial quality is a standalone from GMP compliance. One could be justified either way in a risk assessment, and the other is much more black or white.

Methylisothiazolinone

Methylisothiazolinone and the blend of Methylchloroisothiazolinone and methylisothiazolinone had been restricted for usage only in rinse-off non-sterile product formulations due to contact allergy issues.

In a recent update of EU Annex V-List of Preservatives allowed in Cosmetics, the maximum usage concentration of methylisothiazolinone had been reduced to 15 ppm.

Has anyone notice that this maximum concentration of 15 ppm for methylisothiazolinone is now below the MIC/MLC levels for having biological activity as a preservative?

Because the maximum usage concentration of 15 ppm for methylisothiazolinone is now below the necessary levels for demonstrating biological activity, why is it still on Annex V for usage as a preservative in rinse-off product formulations? Does this make any sense?

A1: The approach taken in EU Annex 5 would appear to make no sense scientifically.

A2: Tho useless by itself at 15 ppm, this does not restrict incidental presence in the combination of MIT with MCIT.

A3: A complete ban would have also banned the otherwise ok use of methylchloroisothiazolinine due to its incidental MIT.

A4: In the SCCS/1521/13 Revision of the opinion on methylisothiazolinone (P94), there is the presence of the following 2 statements:

For rinse-off products, it may be considered that circa 3.8 ppm MI (as in the MCI/MI mixture) is acceptable as this is the amount present when MCI/MI (3:1) is used at 15ppm for preservation of rinse-off cosmetic products, but it is unknown whether this concentration provides useful preservative activity.

For rinse-off cosmetic products, a concentration of 15 ppm (0.0015%) MI is considered safe for the consumer from the view of induction of contact allergy.

From these statements, it can be concluded that the concentration of methylisothiazolinone (3.8 ppm) in a blend of MCI/MI should not be a problem for inducing contact allergy issues.

Upon further investigation, it seems that the concentration of methylisothiazolinone had been reduced due to the results of a recent Scandinavian study that did not support safety of MI in rinse-off products at either 100 ppm or at 50 ppm for the induction of contact allergy. This information is present in the following reference:

Scientific Committee Of Consumer Safety, Giménez-Arnau AM. Opinion of the Scientific Committee on Consumer safety (SCCS) - Opinion on the safety of the use of Methylisothiazolinone (MI) (P94), in cosmetic products (sensitisation only). Regul Toxicol Pharmacol. 2016 Apr;76:211-2. doi: 10.1016/j.yrtph.2016.01.001. Epub 2016 Jan 11. PMID: 26790577.

However, I do not have a reference for this Swedish study.

A5: here is the link to the Swedish Study

https://www.medicaljournals.se/acta/content_files/files/pdf/95/6/4331.pdf

Conclusion

Previously published results from other countries have shown that the contact allergy frequencies to MI are high and increasing with a subsequent increase in also the contact allergy frequencies to MCI/MI. This study confirms that the contact allergy frequencies to MI and MCI/MI are high also in Sweden. This is most probably due to an increased exposure to MI as a result of its introducing in 2005 as a monopreservative itself in cosmetics. An increased use of MI in combination with the fact that no legislative changes regarding the use of MCI/MI has been taken since 1989, indicates that the observed increased frequencies are a result of MI being the primary sensitiser in most cases. With this study as a basis the Swedish Society for Occupational and Environmental Dermatology included MI in water at 2,000 ppm in the Swedish baseline series from January 2014, thus the same concentration/dose as the recommended one for the European baseline series [8]. This corresponds to a dose of 60 µg/cm2 when applying 15 µl with a micro-pipette and using the Finn Chamber® (diameter 8 mm) technique.

Another 2020 Study Link: https://onlinelibrary.wiley.com/doi/abs/10.1111/cod.13483

A6: Thank you for the link to the Swedish paper.

In reviewing this paper, a 2000 ppm concentration of MI and a 200 ppm concentration of MCI/MI were used in this study.

They are saying that a 2000 ppm concentration of MI corresponds to a dosage on the skin at a concentration of 60 ug/cm3.

If they are using the above application dosage on the skin for MI, I can see as to why they are saying that a 100 ppm concentration is not allowed for usage in product formulations as a preservative.

Reduced testing per USP <1112>

We have some cosmetic products that qualify for "reduced testing" due to their low water activity per USP <1112>. For products like these, how often do you perform USP <60/61/62> since lot-by-lot is not required? Quarterly? Yearly?

Thanks for any insight!

A1: The frequency of your skip lot is very much dependent upon both the number of batches you will be producing and the overall level of risk to the product outside of your foundation of low water activity.

Sustained low water activity of a formulation will prevent growth in a sample but it does not work as a biocidal/fungicidal perse.

Your risk assessment shall identify possible addition of contamination during manufacture and production as well as in-use.

If you make over a thousand batches in a year, I'd have to say testing annually would be troublesome.

I generally find it more advisable to put that frequency at a per number of batch level say 1 in 10 batches or 1 in 50 batches but, once again that depends on your overall level of risk.

A2: A historically good practice is 1 of each 5 lots.

A3: Not a big fan of skip lot or annual microbial testing.

Either the risk is mitigated or it is not. If the risk is mitigated and the testing is a compliance sop and it is a non-value added activity. If it may occur occasionally, testing may miss it. If the annual batch has high counts would you test the retention samples for the past year.

ICH Q6 figures 6 and 8 do support water content/water activity in establishing microbial specifications for pharmaceutical ingredients and drug drug products.

A4: [name redacted] this works for a chemical assay with control charting the results.

A5: I'm in agreement with [name redacted] in that I'm also not a big fan of conducting skip lot or reduced testing. There have been many occasions in which I have found products with a low water activity level were contaminated with microorganisms by them being able to survive in it but not proliferate. In addition, I would not just depend on using only water activity to justify the usage of a reduced or skip lot testing program.

I have a question for you. Is your product aqueous or contains water? If not, I suspect that your product may be an atypical personal care or cosmetic product formulation. If your formulation is an atypical

product formulation, I would suggest that you obtain a copy of the PCPC guideline called "Microbiological Risk Factor Assessment of Atypical Personal Care Products" in the PCPC Microbiology Guidelines for further information to determine whether if microbial testing is even required.

There are many personal care or cosmetic product formulations that are considered to be atypical product formulations in which no microbial testing is required for Quality Control release. The above guideline contains information that can be used to justify not conducting microbial testing on an atypical product formulation.

A6: [name redacted], to answer your question, these products are largely petrolatum-based.

A7: I'm also not in the favor to the skip or reduced microbial testing.

Microbiology is not the obvious or definite numbers as chemical but in contrast we perform each and every chemical test for the release and in most companies they do at every stage even they know the 100% API is used for batch manufacturing, but on other hand, every person is talking about skip microbial testing because they give the logic of low water activity, high compressed pressure which puts such pressure during compression to destroy the microbial cells, controlled environment, etc...

I ask a question here, we agree the microbial growth dependent on the water and low water activity not support the growth but what about the spores either bacterial or fungal, when they get the favorable conditions are they not proliferate, compression suppress the microbial cells, but did anyone notice that all of these when we perform microbial test why the growth appears? Why in pharmacopoeia limit is 20, 200, 2000 instead of 10, 100, 1000???

And what about the coated tablets, capsules, syrups???

Everyone talking about ICH guidelines but not talk about 21 CFR Guidelines, in which clearly stated that each and every batch must be tested for microbial growth before release or market.

A8: So I'm tickled somewhat by the responses against Skip Lot Testing that have been given, not least because it underlines an 'out-of-touch' perspective.

Skip Lot testing is a way to be in whilst being out (those friends of cricket may see the potential for humour).

First of all, many of our colleagues are dependent on the necessity of microbiological limits testing.
Secondly, many of our businesses are looking to reduce costs without removing quality.
Thirdly, it is your job to find the balance between staff reductions and irate financial teams.
All whilst maintaining the integrity of your position, your product, and your employer.

That said, Skip Lot testing is not and should never be predominantly a decision made entirely on the benefits of a single parameter.
If your doing that, STOP, because you're doing it wrong.
Nor, it could be said is it ever to represent an over reliance on preservative efficacy.
It is an exercise in application of knowledge, if you don't have it don't do it.
That said, the process will lead to you having a better understanding regarding the life cycle of your products, which in itself is an immediate improvement.

I think to take a perspective of black or white, test or don't is not in any way helpful. Never let go of the ownership of quality on your portfolio.
You need to be able to oversee changes that may impact subsequent reviews and you should be willing to review those initial changes regularly.
That doesn't mean that testing periodically is a half measure of no value, it means that you remain involved in the life of your product with a better understanding of the microbial risk it poses.

If you don't know what the right decision is, or the correct frequency, or whether you should be doing it at all then STOP. You are likely not the right person, or don't have the right information to make that decision.

That said, [name redacted] PDA publication is a good place to start for water activity applications, but the real information is in your own data.

A9: 21 CFR 211,165 (b) There shall be appropriate laboratory testing, *as necessary*, of each batch of drug product required to be free of objectionable microorganisms.

Not an absolute requirement when justified by a risk assessment.

A10: Petrolatum or petroleum jelly is insoluble in water but is slightly soluble in alcohol. Petrolatum is a mixture of hydrocarbons obtained from petroleum. Depending upon the exact proportions of the hydrocarbons being present, the melting point of petrolatum is generally between 40 and 70C. Because of this, I would expect that your product would have a low water activity level. In addition, I would not expect that your product would be susceptible to microbial contamination if the product is nearly 100% petrolatum and fragrance.

Because your product is petrolatum-based, I expect that your product is either a moisturizer or skin protectant. If there is no water in your product, I suspect that your manufacturing process might be using temperatures greater than 65C to make and fill it, resulting in a sterile product if the product is nearly 100% petrolatum. However, I have seen petrolatum products consisting of 30% petrolatum containing water in which I would not recommend that skip-lot testing be performed because they contain a good amount of water with preservatives such as phenoxyethanol and parabens.

In the end, it comes down to the composition of your petrolatum product formulation.

A11: One could justify having a chimpanzee perform their micro testing with a slick enough risk assessment.

Kidding aside, RA's don't give me much confidence here. In firms where "sales" guides the culture over quality, I've seen RA's allow some extremely egregious practices and philosophies. I fully understand the intent. If they are used honestly over 50% of the time I'd be shocked.

Bioreactivity study USP<87>

Anyone having biological reactivity study protocol? Please share. It will be appreciated.

Microbial test method validation

I have the following concern:

If I have one product with more than one label clime (strength). Where the only difference is the concentration of active material. Whatever , it's known that active materials have no antimicrobial activity.

-The question is, is it acceptable to make TMV for only one of the label claims?

- Is there any guideline that discusses this specific matter?

ENDOTOXIN TEST MVD PRODUCT WITH 2 ACTIVES PRINCIPLES

I have a product with 2 active ingredients in its formulation, the endotoxin limit (monograph) is 35 EU/mg.

The question would be for the calculation of the Maximum Valid Dilution, should I use the active ingredient with the highest concentration?

Greetings to all, I hope you can help me, I can't get a monograph on it and the questions and answers from the FDA do not cover this topic.

A1: Actually, I would rather favour the opposite, i.e. the active pharmaceutical ingredient with the lower concentration would be the one defining your MVD. Let us take an example with sensitivity 0.005 EU/mL which is common for LAL and rFC assays:

API1: 1 mg/mL
MVD = Limit * concentration / sensitivity = 35 EU/mg * 1 mg/mL / 0.005 EU/mL = 7000

API2: 0.1 mg/mL
MVD = 35 EU/mg * 0.1 mg/mL / 0.005 EU/mL = 700

In this case, the MVD of the product containing both APIs in the mentioned concentrations would be 1/700. At 1/7000, you would dilute API2 too much to say, if its endotoxin concentration was below its limit.

A2: If the product is a powder, as I imagine from the EL you reported, the value for the C in the MVD formula, depends on the concentration of the stock solution you create starting from your drug in powder

Eg. You have a stock solution 100mg/ml

MVD = (35 EU/mg X 100 ml/ml)sensitivity

It doesn’t matter if the 100 mg are due to 75 mg from API 1 and 25 of API 2

A3: I think there is something not clear in endotoxin limit (EL)definition you proposed

Is 35 EU/mg reported for both APIs or for the DP (drug product)? This is the main point of my doubts

DP = API1 + API2

If it is for an API, I think there is still a problem with the approach proposed by Thomas. It is dangerous considering the EL of a DP taking in account only the EL of a single API. The EL is for the API, not for the DP If you work in this way, you could have no room for an Endotoxin Limit definition for all other raw materials or APIs you have in the DP. Because I think you have to have a check of the raw material and API as per EP 2034 or EP 0520

Second, as for USP 1085, you should always check if the EL you propose is still below the formula EL =k/m

I hope you could agree with my personal point of view on this topic

A4: MVD is based on the dosing of the active drug(s) in the finished product. Two active drugs (2 APIs) would be additive (typically in mg). So TL = K/M where M would be the combined active going into the patient. Then apply MVD = (TL X PP)/lambda. For each API tested alone I would use TL = K/M where M is a single API to determine its own TL and MVD.

Excipients do not enter into this calculation. Each excipient can be calculated taking into account its proportion of the total solution (there are papers about this). Excipients are often tested to be negligible, for example USP limits on common excipients are very restrictive.

A5: For me, I can't answer this question as I'm not clear on the question. There are two Active Product Ingredients, with limits from the monograph, to be used in a Drug Product, or at least that's what I think

you are asking. I need to be clear on how these API are going to be used. Are they part of a final Drug Product? Does that DP have a dosing? Are they combined at time of use? Why are you not calculating from that dosing information for the Endotoxin Limit? I don't think it matters what the monograph limits are on the API as those limits were not calculated with your application of them in consideration. The API are just a part of a whole that needs an EL that should be calculated from the dosing information of the DP. How old is the patient, and what's the route of administration, as well, need to be considered to calculate the EL and then MVD.

Once you are clear on the Endotoxin Limit of the Drug Product then you can calculate MVD to test the Drug Product as a whole. I think that's what you are asking but I'm not sure from your post.

Once you understand this information you might consider backing out the calculations to form better raw material Endotoxin Limits for the API and other raw materials used in the Drug Product. It's entirely possible that the EL from the monographs are not good limits for your usage of the API. I can't say one way of the other as I don't have enough information.

A6: if the monograph gives the limit of the whole ingredient then why do you take high concentration for MVD?
For MVD take the whole ingredient such as for example your product concentration is 100mg/5ml (supposed) so
MVD = *EL x concentration*
Lysate sensitivity(0.25EU/ml
MVD = *35 EU/mg x 100mg/5ml * = 2800
0.25 EU/ml

A7: the product has this mix :

DIPROPIONATE BETAMETHASONE (5 mg) / PHOSPHATE SODIUM BETAMETHASONE (2 mg) / 1 mL

Only Phosphate S. betamethasone has an established limit of 29.2 EU/mg and dipropionate does not have an established limit, the regulatory health entity (in Peru) established a limit of 35 EU/mg for this mixture.

I have carried out endotoxin tests on the raw materials to approve them, and in both cases the endotoxin limit is very low, but we are in the validation phase and I would like to have the correct bases to accept the limit of 35 EU/mL since I do not find an endorsement for this limit.

Vitek 2 bacterial suspension preparation

Does anyone have experience to prepare a bacterial suspension of Bacillus genus for Vitek 2 identification system.

Some of the isolates required to be identified are gram positive rods which by morphological appearance on plates are bacillus genus (ex. Bacillus subitilus , Bacillus cereus , Bacillus amyloliquefaciens and etc.).The problem , I usually face that the culture of some of these bacteria are so dry or mucoid (so sticky) , not readily soluble in water which cause that i am unable to prepare proper bacterial suspension. Vortex and scratching against tube wall may help but I get a lot of unidentified results in case of Bacillus genus.

A1: After you break up the growth on the side of the tube and/or vortex, allow for the suspension to sit for about 20 minutes, gently mix and then pass it through a 40um cell strainer.

This will clear out any large clumps and result in a more uniform suspension.

Retesting of Out-of-Specification Test Results by USP Chapter 61 and 62

In the past, USP Chapter 61 and 62 had a retest section in which double of the amount of the test sample could be performed to confirm an out-of-specification test result but has been removed in revisions of these chapters. It is my understanding that a retest now only could be performed on a test sample if there is evidence that a laboratory error had occurred during testing which caused the sample to be out-of-specification. From my perspective, these laboratory errors could be due to the use of incorrect aseptic technique in conducting the test and/or the use of contaminated microbial count diluents and microbial growth media. If you demonstrated during your investigation that an out-of-specification test result was due to a laboratory error, you are allowed to perform a retest.

I have a question. To perform a retest of an out-of-specification test result due to laboratory error, do you test the original amount of sample or a greater amount of the test sample? I would say that you test the same amount of test sample as in the initial test if the result was due to laboratory error.

In those instances, the initial out-of-specification test result had not been proven to be due to laboratory error during your investigation, do you still fail the test sample since retesting is not allowed or conduct a retest by using a greater amount of sample? When you tell people with a Chemistry or Toxicology background that are responsible for supervising a Quality Control Microbiology Laboratory or the bean-counters that retesting is not really allowed if the initial out-of-specification test result was not due to laboratory error, it is funny to watch them exploded.

Are people conducting a retest in these instances in which the result was not due to laboratory error by using a greater amount of sample since microbial contamination in a batch is never homogeneously distributed? With low levels of microbial contamination (e.g., <100 CFU/gram), I usually find the antimicrobial activity of the preservative system in a product formulation will kill off low levels of microbial contamination by the time you conduct the retest. In addition, I have never seen a preservative system in a product formulation be able to kill off microbial contaminants when the level is greater than 10e6 CFU/gram. How are people handling these types of instances in which the source of the initial microbial out-of-specification test results cannot be determined?

A1: I would refer you to PDA Technical Report No. 80 *Microbial Data Derivation Investigations in the Pharmaceutical Industry* 2022

Retesting a product with twice the sample size, is not an industry practice, or compendial recommendation and is unacceptable to regulatory agencies.

Just one objection is it is unlikely to be a qualified method.

A2: In your scenario you invalidated the original test. So, just repeat with the same amount.

Shaker incubator EM requirements

I'm curious about EM requirement for shaker Co2 incubator which is used for Upstream process (in C grade area).
Currently, I perform EM for microbiology incubators for supporting data purposes of any unexpected situation occurs.

If it should be done also for shaker incubator, which spesification can i follow and which media should be used for EM (because of it is Co2 incubator).

Microbial limit for nutraceutical products

Please suggest for microbial specification limit for nutraceutical products.

A1: See USP Chapter 2023 as a guide.

Environmental Isolate challenge during Method Verification

I am looking for some discussion on which path industry is moving to. There has been an ongoing discussion (or debate) on the use of Environmental Isolates during Method Verification. This is not a recommendation by Compendia Chapters, but more and more observations by Regulatory are coming through in industry for this.

What is the path industry is taking or moving to?

A1: The method suitability requirements are detailed in the compendial chapter.

See Guilfoyle, D. E. and A. M. Cundell 2022 Do Plant Isolates have a Role in Method Suitability and Growth Promotion Testing in the Microbiology Laboratory? Is it a Matter of Science versus Compliance? PDA Journal of Pharmaceutical Science and Technology February 2022, pdajpst.2021.012675; DOI: https://doi.org/10.5731/pdajpst.2021.012675

A2: Please clarify what assays or compendial chapters you are referring to.

A3: Just this week the PDA Journal published an article by Tony Cundell and Dennis Guilfoyle that addresses this topic as well as EM isolates for GPT: PDA J Pharm Sci and Tech 2022, 76 444-460

Their overall argument was that for method validation, yes, plant/EM isolates are useful but not for routine GPT of media. The article is very good and I recommend it for everyone interested in this topic. I personally agree with their conclusion as well....not that it matters.

A4: Yes, Method Suitability requirements are detailed in the Chapter but not for Plant Isolates.

A5: Chapters <51>, <61>, <71>

A6: Yes, no details on plant isolates as they are not a compendial requirement

A7: I expect that this is a bandwagon type observation (“I heard that these should be used, so it MUST be something this firm isn’t doing”).

EM isolates have a place in their usefulness. For growth promotion challenges (EM plates, APS, sterility, etc.) perhaps…. But there’s a few considerations.

* Once you take the wild type strain and preserve it, store it and revive/resuspend it, it is not much different than a “store bought” strain purchased from any manufacturer.
* I would also take a hard look at how different is that EM isolate than any organism that I am already challenging your assays with. If you recover an organism that is unique to YOUR environment and vastly different from your compendial challenge panel, then MAYBE I would take it into consideration.

I can’t tell you how many times I have seen folks perform suitability assays with a half dozen different personnel/operator recovered gram positive cocci microorganisms because they felt they ALL needed to be included. Craziness.

If you already have representative microorganisms demonstrating recovery in your assay, then why the need to expand the panel?

Ultimately it depends on what method/assay you're verifying and which microorganisms you’ve recovered. I am more apt to say it is NOT needed given the narrow bandwidth of species we normally find in our cleanrooms. If you have something that keeps popping up in your EM, that should be an investigation in and of itself.

Don't forget, EM isolates shouldn't wind up in your product if you have a solid control strategy.

I know, a long-winded answer, barely worth more than $0.02!

pH after sterilization

Could you please provide any reference regarding after sterilization media PH.

As per USP General chapter <61> mentioned that after sterilization perform PH at 25 °C. But it is not mentioned any range.

In USP Genral chapter mentioned <1117> that The pH of each batch of medium should be confirmed after cooled to room temperature (20-25 °C) .

In USP Genarl chapter mentioned <791> that pH shall be performed at 25°C +/- 2°c.

There are different statements mentioned in different chapters but below 1000 USP chapters are mandatory requirement to follow.

aPlease share your opinion regarding this.

MLT Culture Suspension

I have a query regarding Microbial culture suspension. In all pharmacopoeias it is clear mention for growth promotion test as follows.

Growth obtained on a solid medium must not differ by a factor greater than 2 from the calculated value for a standardized inoculum.

For a freshly prepared inoculum, growth of microorganism must be comparable to that obtained with a previously tested and approved batch of medium.

For 1 what is the calculated value for a standardized inoculum.

For 2 if i prepare fresh culture suspension and select 10 to 100 cfu dilution and store it. and run tests on fresh mediums. but i have not previously lot of media then how we compare.

Help with USP nitrogen as a transporter

Dear colleagues, I need your help to remember a USP chapter.

In particular this says

“It is unlikely that the nitrogen will be contaminated, it will act as a transporter.”

I need it to support the change in frequency, since we currently sample it in each product packaging and I want to place it once a week, supported by the fact that the nitrogen previously enters through a 0.22 um filter and, in turn, the sampling history in the filter he has a perfect record.

If anyone remembers which chapter I appreciate it since I don't remember exactly the chapter.

Clorox Cleaner Recall

It should be noted that a Clorox Cleaner had been recalled due to the presence of Ps. Aeruginosa. It seems that this is a problem formulation because the same cleaner had to be recalled last year due to the presence of Ps. Aeruginosa contamination.

A1: Yes, 6 brands of Pine-Sol. The ability of these strains of P. aeruginosa should be investigated as Pinol-Sol is registered as active against this bacterium.

A2: How is it possible for a chlorine based disinfectant to contain Ps. Aeruginosa? I'm really surprised. Did they bottle water or something?

A3: I used to test disinfectants before I went to graduate school… I am surprised they released them to the market!

A4: One more time this strain has proved its ability to resist and grow in presence of yet another active ingredient. Worth looking into if the active levels or other boosting excipients in the formula were reduced or substituted as a part of cost cutting.

Another big lesson- THERE IS NO SUBSTITUTE FOR GMPs.

A5: Perhaps you are confusing Clorox brand Pine-Sol cleaners with Clorox brand bleach. Pine-Sol uses different chemistry than their “famous” bleach (sodium hypochlorite).

The recalled product is a preserved formulation that utilizes Glycolic Acid as a disinfectant with several surfactants.

A6: Agree - this is another example of very poor manufacturing hygiene. Wonder if they content tested finished product before release. Almost any product can be contaminated - including disinfectants and even 70% ethanol (with cepacia). I recall a contaminated (aeruginosa) quat disinfectant - the bug was traced to the quat active itself.Troublesome that the same issue was encountered in their Peruvian market almost 2 years before this one.

https://journals.asm.org/doi/full/10.1128/AAC.00138-07https://www.researchgate.net/profile/Ziad-Daoud/publication/8640901_Outbreak_of_Burkholderia_Cepacia_Bacteremia_Traced_to_Contaminated_Hospital_Water_Used_for_Dilution_of_an_Alcohol_Skin_Antiseptic/links/00d69629cc126956e1efa27f/Outbreak-of-Burkholderia-Cepacia-Bacteremia-Traced-to-Contaminated-Hospital-Water-Used-for-Dilution-of-an-Alcohol-Skin-Antiseptic.pdf

A7: At one time, Pine-sol cleaner did contain Pinus palustris (long leaf) oil as the active ingredient. In 2013, Glycolic acid was used instead as the active ingredient in the product to replace the Pine oil in order to reduce the cost of the product. It is funny that the name of the product is Pine-sol, but does not contain pine oil.

It is my understanding that glycolic acid is suppose to have antimicrobial activity against Ps. aeruginosa. It is also my understanding that some people have used glycolic acid as a preservative in product formulations.

For Ps aeruginosa to survive in this recalled product, I suspect that the survivability of Ps aeruginosa is due to either some type of a biofilm issue in which Ps aeruginosa is protected from the antimicrobial activity of glycolic acid or there is a pH issue with the product that is above a pH of 4 to cause antimicrobial neutralization of glycolic acid.

It would be interesting to see the results of root cause investigation of why the product is contaminated with Ps. aeruginosa.

A8: Your history of the pine-Sol formulation, as are your thoughts on effectiveness against gram negatives are accurate.

My point was solely that this particular product is not (nor has ever been) a chlorine-based disinfectant.

A9: Nothing survives bleach. However about 10 years ago a client found Bacillus spores that did survive. Needless to say I was quite scared since bleach is the last resort for destroying microorganisms. I'm not sure what happened to the bug but I do know that the client site still exists - hopefully without zombies running it.

A10: Some fungi associated with bathroom mildew produce multicellular melanized structures that survive immersion in bleach.

A11: It is telling that Pine-Sol is not included on the EPA List G of registered disinfectants so should be viewed as a cleaning agent, which why the product was subject to Consumer Product Safety and not FDA recall.

A12: In regards to your comment, I will say that in Microbiology that you can always find an exception such as being able to find viable spores to be present in bleach. Generally, Sodium hypochlorite in bleach has sporicidal activity because chlorine is able to remove protein from the spore coat that has an adverse effect on spore germination. In the literature , analysis has indicated that Bacillus spores are more resistant to chlorine than Clostridium spores. Go figure. I find this to be strange because I was always taught that Sodium hypochlorite was 100% sporicidal against all spores. In these types of cases, you just have to be careful of the zombies that may be present in a facility where bacterial spores are able to survive in bleach that is used to make a chlorine disinfectant solution.

A13: Even scarier than Bacillus spores. Fungus among us.

Bioburden Rinsate

USP <61> calls for the use of Phosphate buffered solution or *Buffered Sodium Chloride–Peptone Solution pH 7.0 as a rinsate for bioburden using the membrane filtration method.*

* <71> calls for the use of Fluid A as a Rinsate for membrane filtration method for Sterility. Can anyone tell me why Fluid A is acceptable for sterility testing, but not for bioburden? I have seen a few labs recently getting audit observations for using, and verifying the bioburden method with Fluid A as a rinsate. *

A1: This is a bit bizarre...USP 61 even mentions TSB as an option. Maybe it's an inspector who "doesn't see it listed" in the compendia? I suppose if a firm wanted validate any of these basic peptone solutions for a rinsate, it would be fine from a microbiology standpoint.

A2: Fluid A (peptone water) is used for diluting or rinsing when performing sterility testing or Bioburden testing. Fluid D (peptone water with poly-sorbate 80) is used for diluting or rinsing samples containing lecithin or oil when performing sterility testing or Bioburden testing. Meets United States Pharmacopeia (USP) performance specifications.

I can think of inclusion of poly-sorbate 80 as neutralizing agent in Fluid D which act as Neutralization of Antimicrobial Preservatives in the product sample.

A3: In could be due to the fact that Fluid A is 0.1% Peptone that is used in a membrane filtration method in USP 71 in which the antimicrobial activity of the product is neutralized away by the product passing thru the membrane filter and rinsing the filter. It might not be sufficient to accomplish antimicrobial neutralization of preservative systems in non-sterile products in USP 61 enumeration method. By changing the pH of a product sample by using Buffered NaCl Peptone Solution as the diluent , it may accomplish neutralization of organic acid preservative system in a product formulation which is not possible by using Fluid A. I have found that people will incorporate 4% Tween and 0.5% Soy Lecithin in

this diluent because TSB is not able to neutralize the preservative system that is present in a non-sterile product formulation.

A4: I agree with your assessment, however, I was specifically referring to the bioburden method by membrane filtration. The principles would be the same for a bioburden sample in that the antimicrobial activity of the product is neutralized away by the product passing through the membrane filter and rinsing the filter. Essentially, I am trying to understand:

Is there a scientific rationale for omitting Fluid A as a rinsate for the membrane filtration bioburden method in USP <61>?
Is it acceptable to use a rinsate that is not specifically mentioned in USP <61> for bioburden testing, provided that the risate has been qualified?
Or, if a bioburden method uses a rinsate not specified in USP <61>, Is this method now considered "non-compendial" which would require a full validation?

A5: Trying to answer:

Q. Is there a scientific rationale for omitting Fluid A as a rinsate for the membrane filtration bioburden method in USP <61>?

A. Tough one to answer, considering USP <61> was written many years ago and the expert committee at the time would know why.

Q. Is it acceptable to use a rinsate that is not specifically mentioned in USP <61> for bioburden testing, provided that the risate has been qualified?

A. Yes. Start with USP <61> and if this does not work for you then you can qualify a more appropriate rinsate. “If growth is inhibited (reduction by a factor greater than 2), then modify the procedure for the particular enumeration test to ensure the validity of the results. Modification of the procedure may include, for example, . . . incorporation of a specific or general neutralizing agents into the diluent.”

Q. Or, if a bioburden method uses a rinsate not specified in USP <61>, is this method now considered "non-compendial" which would require a full validation?

A. See above. If you are modifying the existing rinsates to meet the requirements for neutralization, as per USP, then you would not have to validate a new test (i.e., the only thing you are changing is the rinsate). See Table 2 for examples of neutralizers that may be added.

A6: Here's my take on the topic.

To start, I do not agree that using Fluid A as a membrane filter rinse is observation-worthy in an audit. If it does come up in an audit and progresses to an observation then I assume either the explanation by the lab was very poor or the auditor is the type that knows enough microbiology to be trouble, but not know enough microbiology to be A LOT of trouble. Here are my reasons:

#1 The membrane filtration section of the USP MLT test chapter does not specify the identity of the rinse as phosphate buffer or anything else. The text reads: "... rinse the membrane filter with an appropriate volume of diluent." That's it.
#2 In the USP MLT test chapter section "Recommended Solutions and Culture Media", the text reads: "The following solutions and culture media have been found satisfactory for the purposes for which they are prescribed in the test for microbial contamination in the Pharmacopeia. Other media may be used provided that their suitability can be demonstrated." That last sentence is important.
#3 The true requirement (in the US at least) is whether or not the practice of using Fluid A as a rinse satisfies 21 CFR §211.194 which says a method shall be verified under actual conditions of use. If you've properly performed method suitability using Fluid A as the rinse, then you have data demonstrating the method is suitable under actual conditions of use.

I wonder if there is more to this story. For example, perhaps the contract lab performed method suitability on a particular test material using phosphate buffer but switched to Fluid A without repeating method suitability. I've submitted method suitability data to FDA for review many, many times. Some methods even used Fluid A as a filter rinse. I've never had an FDA reviewer object to or question why a certain medium was used as the rinse if the data supports it.

These are my opinions, so others may disagree.

A7: Fluid A has greater properties than phosphate and sodium chloride, it has the same properties as a general rinsing buffer and, very importantly, it is compatible with most samples, it is used to dissolve or dilute samples, reconstitute microorganisms and transport medium of microorganisms.

I remember that we had a product that could not be treated with phosphate because it reacted, we did not change to sodium chloride but to peptone water to provide stability and growth properties.

Aerococcus species

Are people isolating the presence of Aerococcus species in their microbial air samples of non-sterile manufacturing environments?

I know that Aerococcus is a Gram-positive cocci that can be present in air and dust samples. In the past, I do not recall in isolating this genera in air samples, but now I'm able to identify it as being present in air samples from time to time from different manufacturing facilities. I just wondering whether that we now have better identification technology (e.g., 16s rRNA) today in comparison to what we had used in the past that is one of the reasons as to why it is now being detected. The other reason is that it was always present in a facility, but only Gram-negative isolates obtained in air samples were only identified to the genus/species level and the rest were only identified as as Gram-positive coccus or bacilli isolates by using only the Gram stain.

Are people conducting biochemical identification of isolates obtained in air samples now on a routine basis in non-sterile manufacturing facilities?

Reference Microbial Culture

As per USP FAQ (Chapter 61 and 62) You should use the strains that are cited in General Chapter <61> or equivalent strains from other culture collections. For example, if Pseudomonas aeruginosa ATCC 9027 is indicated, you should use this strain or strains from other culture collections claiming equivalence to ATCC 9027. Other strains such as Pseudomonas aeruginosa ATCC 14149 are not appropriate.

Can I know why we can not use other Pseudomonas aeruginosa strains? While Pseudomonas aeruginosa ATCC 9027 is used for GPT.

What is the criteria for equivalency? If we isolate Pseudomonas aeruginosa and show characteristics similar to ATCC 9027, can we use it?

A1: The global culture collections take organisms from all different sources and we try to normalize our very un-normalized microbiology testing by taking one variable out of the equation, different strain types. As an example, when I was working at a Biolog, we had a really good database but it still didn't include every organism in every culture collection, obviously. At that time, Enterococcus hirae had only 2 strains in the ATCC culture collection. For some reason, our customer bought the other one, not the type strain. It had a totally different phenotypic profile and they got "No ID". Wondering why an "ATCC strain" wouldn't work on our system, I looked into the strain they bought and it turned out to be a research strain with genetically altered traits, resulting in a totally different phenotypic profile. I don't think it would be wise to use an in-house isolated Ps. aeruginosa as a substitute for the ATCC type strain.

A2: I want to know why Escherichia coli ATCC 8739 or equivalent culture for example NCTC 12923, MTCC 1687 or any other strain form culture collection centre is used as a reference culture for microbial examination of non sterile products. What is the story behind this? Why we can not use other Escherichia coli strains and why this particular strain is used for microbial examination of non sterile products.

The above mentioned strain number from different collection centres is equivalent to ATCC. For this there is only one depositor who deposits their culture in different gene banks or deposits in one gene bak.

Or it may be other depositors who isolate their own culture and show equivalent to ATCC.

I noted on NCTC website they show an equivalent culture to ATCC in list and type strain show "NO" and "YES". If yes it means that genus and species are the same. What is the meaning of "NO" and if type strain no how he show equivalent to ATCC.

A3: The story is that these are the most characterized, stable strains that we have in our culture collections. We settled on organisms that have been studied for decades and have (mostly) repeatable performance. That's it. If you'd like to see the history of ATCC, you may go to their website to see how the started. USP and ATCC are North American based companies so it only makes sense for them to work together to make the best decision.

A4: Coincidentally I am presenting on this topic at Pharmig this week…

The USP Chapter <1117> says “Cultures for use in compendial tests should be acquired from a national culture collection or a qualified secondary supplier ▲and have documented equivalency to relevant ATCC strains (2).”
Reference 2 is “Reference Strain Catalogue Pertaining to Organisms for Performance Testing of Culture Media (v 29)"

* It provides “a unique system of identifiers for strains recommended for use in quality assurance”
* It links the strain numbers of the World Data Centre of Microorganisms (WDCM) to other sources of the reference strains
* Helps users find local sources of the reference strains by citing many collections
* Provides collection acronyms, contact details, and each collection’s unique strain numbers

A species is composed of the type strain plus all other strains considered to be sufficiently like the type to justify inclusion with it in the species.
The very first strain published and characterized is defined as the “type strain” and is the permanent name bearer and the original reference specimen for the name.

Note: Pseudomonas aeruginosa ATCC 9027, CIP 82.118, NBRC 13275, or NCIMB 8626 are listed in the USP/Ph Eur documents – and is NOT the type strain for the species.
Pseudomonas aeruginosa Type strain is: ATCC 10145; ATCC 10145-U; CCEB 481; CCUG 28447; CCUG 29297; CCUG 551; CFBP 2466; CIP 100.720; DSM 50071; IBCS 277; IFO 12689; JCM 5962; LMG 1242; NBRC 12689; NCCB 76039; NCIB 8295; NCIMB 8295; NCTC 10332; NRRL B-771; RH 815; VKM B-588.

There is the <USP 1117> criteria for documenting equivalency to the ATCC compendial strains. The easiest way to do that if you are not using the ATCC culture is to use the same strain deposited in another global culture collection whose number is listed and traceable in the WDCM tables. If you isolate a “Pseudomonas aeruginosa” strain, you could have quite a burden showing equivalent characteristics to the compendial strain ATCC 9027.

WRT the E coli… ATCC 8739 is the same as NCTC 12923. They are the same strain deposited into different culture collections.
WDCM 00012

ATCC™ 8739; BCRC 11634; CCM 4517; CECT 516; CGMCC 1.2463; CICC 10302; CIP 53.126; DSM 1576; IFO 3972; IMET 11121; LMG 8063; NBIMCC 3397; NCDO 904; NCIMB 8545; NCTC 12923

They may not have the exact same characteristics as the type strain for E.coli which is ATCC 11775; CCUG 24; CCUG 29300; CIP 54.8; DSM 30083; JCM 1649; LMG 2092; NBRC 102203; NCCB 54008; NCTC 9001. That is why they are not interchangeable. As Tim said – normalizing the strains recommended by the harmonized pharmacopoeia chapters for microbiology testing takes one variable out of the equation.

The NO and YES on web pages (or check marks) is indicating whether that strain (deposited into many global collections) was the first strain published and is defined as the type strain for the species. See BacDive/LPSN:
https://bacdive.dsmz.de/strain/4433
https://bacdive.dsmz.de/strain/4907
https://lpsn.dsmz.de/species/escherichia-coli

A5: Interesting interpretation of USP. However it is contradicted by the expectation to use in house cultures for growth promotion, DET studies, AET studies, etc. Normalizing the bugs is important but why are in house strains now expected?

A6: There is a recent publication in the PDA journal regarding the need, or lack thereof, of using local isolates. However, in a past life, I was involved in an investigation in which a bug grew out in a preserved product. It was a fairly rarely seen isolate in terms of routine EM. We were able to show that it was, in fact, resistant to the preservative system.

Here is the reference;
Do Plant Isolates Have a Role in Method Suitability and Growth Promotion Testing in the Microbiology Laboratory? Is It a Matter of Science versus Compliance?
Dennis E. Guilfoyle and Anthony M. Cundell
PDA Journal of Pharmaceutical Science and Technology September 2022, 76 (5) 444-460; DOI: https://doi.org/10.5731/pdajpst.2021.012675

A7: I also have experience in which a bug grew in a preserved product. By conducting MIC/MLC testing on the bug, we found that the preservative system concentration was below the MIC/MLC levels for the bug. We also never found it to be present in any of the environmental samples. Sometimes,it is very difficult to correlate out-of-specification test results for finished products with environmental test data.

A8: You are preaching to the choir 😊 As in, "It must be the lab. We didn't find it in the settling plates for that batch"

Next sound is me hitting my head on the desk.

A9: I wish that I had a nickel for everytime that manufacturing stated that an out-of-specification test result for a batch was the fault of the lab. I would be rich. I also know the feeling of hitting my head against the desk in these types of instances.

Acceptance criteria for Total count

Can you tell me what is the acceptance criteria for duplicate plates in total count for non sterile products? how much variation is acceptable for duplicates plates.

A1: USP 61 states to average the two plate counts (pour/spread) so I don't see any limit on counting CFU between duplicate plates with non-sterile samples. If you're using membrane filtration, you'll only use one filter/counting per medium so there is no averaging.

In general, if you've performed your dilutions and/or mixing correctly, you should come out with comparable results but even with our known QC growth promotion organisms, we give a factor of 2 for the variability in microbiology testing.

Microbiology...where most tests mostly work most of the time 😊

Reduced testing quantity for DPI capsule product

We have a new product/project we are working with, and the customer is asking if there is any way to justify reduced quantity for USP <61> testing. I am familiar with the "amount used for the test" section of USP <61> and allowance for reduced quantity in specific situations, however I don't feel this product fits any of those scenarios and I was curious if anyone else has justified reduced sample quantity for a similar type of product.

This is a dry powder inhalation product, where formulation is filled into a capsule and one capsule each goes into a single use device. The batch size is in the range of 50,000 capsules, and the amount of active is not less than 1 mg per dosage unit. Testing the bulk filled capsules is not a concern as 10 grams would be a relatively small quantity, the concern is testing the final product with capsule in the device. With a fill weight of 25 mg (plus capsule weight), it would require sampling a couple hundred devices in order to get the 10 gram minimum. I'm not sure how to get around this, even going with an approach of 1% batch size would still be a large number, although devil's advocate could make the argument that for sterility, after a certain point, the number of dosage units can be extremely small compared to total batch size (different test and circumstances, but still), so why could we not take a similar approach for <61>.

A1: Inhalation products are problematic as you pointed out because each unit has such a minute amount of drug product. For this reason there is USP <610>, specifically the Sample Size Determination Section, which provides alternative sample instructions for this type of drug product.

A2: I had remembered reading that chapter a while ago and forgot it included inhalation products. Glancing through it just now, it seems that is exactly what I needed.

Water Bioburden Hold Time in WFI

I am wondering if anyone has designed/executed a hold time study for bioburden testing of water. USP currently states a hold time between sampling and testing at 24 hours and we would like to change it (from our current, which is 12) but need to prove it.
Issues keeping organisms alive in WFI samples has been quite the challenge. We use R2A and a 20-25C incubation for routine testing.
Any suggestions?

A1: Use R2A at 30-35 deg C for 5 days.

A2: You can change the hold time to 24 Hrs based on the recommendation of the USP general chapter.

Since USP has been already Validated the hold time, hence further study is not required.

A3: No, USP <1231> is a general informational chapter not a general test chapter so it does not imply that the hold time is validation.

However, APHA/AWWA Standard Method 9215 Heterotrophic Plate Count is a validated standard method so the recommended, hold time does not need to be validated.

A4: Agreed. USP <1231> is informational, not mandatory. However, in 1231 it reads (section 8.5.1.) "… it is best to test the samples as soon as possible. If it is not possible to test the sample within 2 hrs of collection, the sample should be help at elevated temperatures (2°-8°) and tested within 24 hrs. In situations where 24 hrs is not possible (such as using off-site contract labs), it is particularly important to qualify the microbiological sample hold times and storage conditions to avoid significant changes in the microbial population during sample storage."

I would consider the phrase "it is particularly important to qualify the microbiological sample hold times and storage conditions.." to be critical.

A5: PDA Technical Report 69 is a good document that talks about bioburden and hold times. Also, Sanofi gave a presentation at PDA Micro in 2018 regarding this topic that also may help. It's also a good reminder to everyone to not confuse in-process hold times with sample hold times which are two different measurements.

BET Sampling

According to some technical texts and guidances, finished drug product units may be pooled into a composite sample and assayed; pooling is often mentioned to be accepted for small-volume parenterals (≤100 mL) my question is:
FDA suggests pooling no more than 3 units per composite, is this correct for all productos from 1 - 50 mL?
How could we perform the tests in Large-volume parenterals (>100mL)?
Our company is currently manufacturing both SVPs and LVPs, our LVPs lot number is up to 7500 litres... how could we assure a good sampling and correct BET?

A1: See the 2012 FDA Guideline here:
https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-pyrogen-and-endotoxins-testing-questions-and-answers
pretty clear and cites some exceptions to the rule.

A2: About your questions:

- FDA suggests pooling no more than 3 units per composite, is this correct for all productos from 1 - 50 mL?

FDA Q&A mentioned by Kevin is pretty clear about that.

- How could we perform the tests in Large-volume parenterals (>100mL)?

FDA Q&A states something that is clear in terms of pooling. About sampling, in USP 1085 you can find the statement below:

“Historically, the sampling scheme for [?]nished drug products is to take at least 3 units representing the beginning, middle, and end of the batch. However, for …..or large-volume parenteral, 3 units may not be a representative sample and may identify only those lots that are uniformly and highly contaminated. Sampling schemes should be justi[?]ed and should be based on the known variability of the process,…etc, etc.…

At the end this is the same sentence you could find in the OLD FDA guideline of 1987 (withdrawn)

The sampling technique selected and the number of units to be tested should be based on the manufacturing procedures and the batch size. A minimum of three units, representing the beginning, middle, and end, should be tested from a lot.

B cepacia Product Recall for Laundry Detergents and Household Cleaning Products

It seems the US Consumer Products Safety Commission has just issued a product recall for the presence of Burkholderia cepacia complex, Klebsiella aerogenes and Pseudomonas species in various laundry detergents and household cleaning products.

https://www.cpsc.gov/Recalls/2023/The-Laundress-Recalls-Laundry-Detergent-and-Household-Cleaning-Products-Due-to-Risk-of-Exposure-to-Bacteria

A1: Expect to see much more of this as the "green" revolution rolls on. With the vilification of commonly used preservative systems and their replacement with "environmentally friendly" but relatively ineffective green preservative systems, microbiologists are going to be very busy in the future.

While this has been commonplace in commercial and household/industrial products, we are now starting to see new replacements for the legacy preservatives in drug systems. Expect these trends to continue to the unfortunate detriment of public health.

A2: A consideration I've been thinking about for some time.
As is known, the USP preservative efficacy test is harmonized with the EP test only for the execution methods, but the interpretation tables of the results are much more permissive than the EP ones. I wonder how much weight this has on trade recalls, which seem more frequent in the US than in Europe.

Another interesting point is to understand if after the harmonization of the USP with the TAMC/TYMC counting tests and with less protected products than the European ones, the US market still manages to return within the release limits.

A3: A consideration I've been thinking about for some time.
As is known, the USP preservative efficacy test is harmonized with the EP test only for the execution methods, but the interpretation tables of the results are much more permissive than the EP ones. I wonder how much weight this has on trade recalls, which seem more frequent in the US than in Europe.

Another interesting point is to understand if after the harmonization of the USP with the TAMC/TYMC counting tests and with less protected products than the European ones, the US market still manages to return within the release limits.

A4: It should be noted that most laundry detergents have a very high alkaline pH which would be hostile to the survivability of most organisms. Because of this, I suspect that preservatives would not be included as an ingredient in these types of high alkaline product formulations. See ISO Document 29621. Furthermore, most preservatives are inactivated at high alkaline pH's. I also believe that USP purified water is not used in the manufacturing of laundry and household cleaning products. Tap water is mostly used in the manufacturing of these types of products. I would not be surprised that the root cause for this microbial contamination was the water that had been used to manufacture these recalled green product formulations.

When it comes to the development of so called green products, it can be more difficult to manufacturer and preserve them to be free of microbial contamination especiialy if you do not understand the chemistry behind the non-green product formulations which are being replaced by the green formulations.

A5: Just wanted to add ... a member of the genus Halomonas, (which grow at alkaline pH values) has been isolated from a household product with a pH of 9.0 - 10.5 ... reference, Halomonas alkalicola sp. nov., isolated from a household product plant; Int J Sys Evol Micro 67:1546 - 1550.

Microbial growth in alkaline consumer products

Just wanted to add ... a member of the genus Halomonas, (which grow at alkaline pH values) has been isolated from a household product with a pH of 9.0 - 10.5 ... reference, Halomonas alkalicola sp. nov., isolated from a household product plant; Int J Sys Evol Micro 67:1546 - 1550.

A1: To [name redacted] observation - liquid laundry detergent as well.

Identification of Objectionable Microorganisms

This is to clarify that is there any mandatory requirement for identification of characteristic/non characteristic growth obtained on a selective agar from a non sterile formulation.

We are confirming characteristic growth obtained on any selective agar using biochemical tests. (for example: on MSA , yellow colonies without zone are observed in a non sterile formulation) USP states to confirm further through biochemical tests. We performed a coagulase test and found it to be negative and released the batch. Is it mandatory to identify the organism?

Similarly in case of water if any brown colored colony is observed on Mac Conkey Agar, it is simply non characteristic. is it mandatory to identify as per USP.

Further if it is mandatory, upto what level it should be identified morphology, genus level or species level for a low risk solid OSD non sterile tablet formulation plant where we test each incoming lot of RM (Active and Excepient) and each finished batch manufactured.

Any suggestions and comments with respect to USP is awaited.

A1: I'm currently taking an online USP refresher course that discusses all the micro chapters related to nonsterile testing and this situation was specifically addressed. The regulatory requirement (21CFR211.165(b)) is that we ensure our products are free of "objectionable" organisms and not just free of the specified USP indicator organisms. If you don't identify the growth to at least the genus or ideally species level and perform a risk assessment for your material/product then it is more difficult to say what grew on the plates isn't objectionable. The question is, does your company feel comfortable defending that you believe your unspecified salt tolerant, mannitol fermenting, coagulase negative organism (presumptively not S. aureus but likely another staph species) is acceptable in your product? A genotypic ID to the species level with a species specific risk assessment for the product(s) affected is the better option.

Of course we could also mention that per <1111> the minimum testing required for the products you describe is <61> and absence of E. coli. The test for S. aureus is more critical for topicals, inhalants, and vaginal products.

A2: Generally, low enrichment or microbial count levels of Staphylococcus coagulase negative cocci is not objectionable in non-sterile products. However, precautions need to be used when determining whether a Gram-positive cocci that is coagulase negative isolate is objectionable or not objectionable. In the case of using presumptive identification results such as a Gram-positive coccus being salt tolerant, mannitol fermenting, and coagulase negative, it could lead to problems. My justification for this statement is that there are literature reports of some atypical strains of Staphylococcus aureus isolates that are coagulase negative. The references for this are as follows:

Fontana, C., Cellini, L., and Dainelli, B. (1993). Twelve aberrant strains of Staphylococcus aureus subsp. aureus from clinical speciums. J. Clin. Microbiol., 31 (2), 2105-2109

Fox, L.K., Besser, T.E., and Jackson, S.M. (1996). Evaluation of a coagulase-negative variant of Staphylococcus aureus as a cause of intramammary infections in a herd of dairy cattle. JAVMA, 209 (6), 1143-1146

Akineden, Ö., Hassan, A.A., Schneider, E., and Usleber, E. (2011). A coagulase-negative variant of Staphylococcus aureus from bovine mastitis milk. J. Dairy Res., 28: 38-42

You have to also realize that there are also several other Staphylococcus species being Coagulase positive such as Staphylococcus delphini, Staphylococcus hyicus, Staphylococcus intermedius, Staphylococcus lutrae , and Staphylococcus pseudintermedius and Staphylococcus schleiferi subsp. Coagulans. References for this are as follows:

Varaldo, P.E., Kilpper-Balz, R., Biavasco, F., Satta, G., and Schleifer, H.K. (1988). Staphylococcus delphini sp. Nov., a coagulase-positive species isolated from dolphins. Int. J Syst. Bacteriol., 38 (4), 436-439

Devriese, L.A. (1977). Isolation and identification of Staphylococcus hyicus. Am. J. Vet. Res., 38 (6), 787-792.

Foster, G., Ross, H.M., Hutson, R.A. and Collins, M.D. (1997). Staphylococcus lutrae sp. nov., a new coagulase-positive species isolated from otters. Int. J. Syst. Bact., 47 (3), 724-726

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It is possible that the presence of a coagulase positive Gram-positive cocci that is not Staphylococcus aureus would not be objectionable in a non-sterile product. If you are going to determine whether an isolate is objectionable or not objectionable in a non-sterile product, it needs to be identified to the genus/species level by doing a biochemical identification or 16s rRNA sequencing from my perspective than by just using presumptive identification tests. Furthermore, no matter if an enrichment isolate shows either typical or atypical reactions on a selective/differential agar from USP Chapters 60 and 62, it should be identified to the genus/species level to determine whether they are objectionable or not objectionable.

A3: The members of the 2020-2022 USP Microbiology Expert Committee were well aware of the limitation of the methods in <62> and how the USP does not address screening for objectionable microorganisms. Of all the limitations of <62> recommending the coagulase test for the identification of S. aureus is not one of them.

<62> reads the following:

Staphylococcus aureus Sample Preparation and Pre-Incubation Prepare a sample using a 1 in 10 dilution of not less than 1 g of the product to be examined as described in Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests 〈61〉 <https://online.uspnf.com/uspnf/current-document/1_GUID-0392F79D-1F8A-4B8D-BEC8-C6FD7B39966F_1_en-US>, and use 10 mL or the quantity corresponding to 1 g or 1 mL to inoculate a suitable amount (determined as described under *Suitability of the Test Method*) of *Soybean–Casein Digest Broth*, and homogenize. When testing transdermal patches, filter the volume of sample corresponding to one patch of the preparation (see *Transdermal Patches *under *Preparation of the Sample* in Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests 〈61〉 <https://online.uspnf.com/uspnf/current-document/1_GUID-0392F79D-1F8A-4B8D-BEC8-C6FD7B39966F_1_en-US>) through a sterile filter membrane, and place in 100 mL of *Soybean–Casein Digest Broth*. Incubate at 30° to 35° for 18 to 24 hours. Selection and Subculture Subculture on a plate of *Mannitol Salt Agar*, and incubate at 30° to 35° for 18 to 72 hours. Interpretation The possible presence of *S. aureus* is indicated by the growth of yellow or white colonies surrounded by a yellow zone. This is confirmed by identification tests. The product complies with the test if colonies of the types described are not present or if the confirmatory identification tests are negative.

I look forward to the day when the USP provides nucleic acid-based identification test methods.

USP 797 STERILITY UPDATE

Just wondering if anyone happens to know why the maximum batch size for all CSPs requiring sterility testing was limited to 250 final units in the 2022 USP 797 revision. Is this due to a microbiology-based evaluation on sterility of CSPs or did this derive from aspetic processes/somewhere else? Curious to understand the justification behind this.

A1: The expert committee outlines their rationale in the FAQs, which you can access on the website. https://go.usp.org/2022_Revisions_795_797

The most simplistic explanation for limiting the batch size is that 503As are not manufacturers. They lack the necessary quality systems, contamination mitigation strategies, and process validation. And even if they had these things in place, most state Boards of Pharmacy do not have the expertise to inspect these locations for CGMP-like operations.

A2: See the Sterility Testing section in the following document: https://compoundingtoday.com/NewsletterImages/DePasquale_797_Comments.pdf

A3: Interesting read. I had just asked [name redacted] about the justification for facilities with automated filling lines, if the concerns are manual manipulation and contamination. Curious to read further information on similar cases.

Water system Re-Qualification

We want to relocate the Potable water plant after relocation, What are the validation steps required to perform..

In the purified water distribution loop I want to add a few more user points by extending the loop..what are the qualification activities required.

A1: This appears to be a significant design change, especially for your distribution loop. Plan on repeating your PQ 1, 2 and 3. Michael

A2: I'm also in agreement with [name redacted] that you need to repeat the Performance Qualification for the system. The reason is that you are increasing the overall length of distribution loop for your system by adding additional use-points. In addition, you need to verify as to whether the current pumps of the distribution loop are more than sufficient to quarantine an adequate flow of water in the distribution loop to prevent biofilm formation.

If not, you will need to have a larger pump for the distribution loop which could cause an increase in the purified water temperature in which a heat exchanger may now be required if you are running an ambient temperature purified water distribution loop. There are alot of system design issues now in play by increasing the length of the distribution loop. You might also need to repeat part of the Operational Qualification of the system if new larger pumps are installed.

Sterility test samples requirement

I need to know total no. of samples required for Sterility analysis of prefilled syringes having fill volume of 0.4 ml. Provide relevant reference also.

A1: See Table 2 and 3 in USP <71> Sterility Tests

A2: Table 2 & 3 of USP <71> are confusing. It should use 40 syringes or total syringes equivalent to 40 ml.

A3: It can be confusing, but it is a critical thing to be mastered for sterile manufacturing.

First, understand that you need to meet your batch size requirements first. Then, move to the requirements based on your LABELED fill claim, not your overfill inclusive.

Where you have a less than 1mL fill, you will need to ensure sufficient articles to meet the requirement.

Remember too, your method suitability must cover your maximum batch size and be informed by your routine testing requirements.

Take a few to understand this chapter!

A4: We have a batch size of more than 20000 syringes and fill volume is 0.4 ml. In this case we have to use 40 syringes or the number of syringes equivalent to 40 ml i.e 100 syringes.

[image: image.png] As per this clouse, we need to take 20 samples for testing for each medium.

[image: image.png] As per point no. 1. In the above clause, it needs to take 40 containers, because we have to filter the whole content. So the no. of articles for both medium will come 40. But as per point no. 2, it is written that not less than 1 ml content to be filtered. So if we have to consider a minimum 1 ml sample for filtration, then the total volume will come to 40 ml. i.e. 20 ml for each medium. Now I want to clarify, that i should consider 40 containers for testing or 100 containers, containing total volume 40 ml.

A5: From Table 3: Since your batch size is greater than 500 containers, you need to use 2% or 20 containers to be tested in EACH medium, whichever is less. 2% of 20,000 = 400, so you would choose the lesser amount, or 20 containers in EACH medium. This means the contents of 20 containers must go into TSB and FTM. How much product that goes into each medium will depend on the fill volume, and the requirements in Table 2.

From Table 2: For fills less than 1 mL, the ENTIRE contents of each container is tested in EACH medium. Your fill volume is 0.4 mL.

Therefore, for each batch of product, you will require 40 containers for the sterility test: the entire contents of 20 containers will go into TSB, and the entire contents of the other 20 containers will go into FTM.

NOTE: you can POOL the contents from all syringes and filter this total volume, as long as half of the total volume goes through one membrane and into TSB and half of the total volume goes through a second membrane and then into FTM.

A6: These tables can be confusing, if you work in a mass distribution pharmaceutical company you will always have large batches.

1 STEP
In this case, always see table 3 first, which tells you how many units to take according to the lot size.

2 STEP
Table 2 specifies the volume to use, here it specifies the minimum volume of each bottle to use, I give you an example if you have a bottle of 100 ml or more, you must use 10% or a minimum of 20 mL.

In your case the volume is less than 1 mL you must use the entire content.

In particular, I use 100% of the volume, so I have it validated.

In your case and due to the small volume you should use 40 units. If you had a larger volume you could use 20 units using the minimum volume.

Neutralization of Fruit Punch Flavor

I am currently working on method suitability for a powdered fruit punch flavor that contains approximately 70-74% Arabic gum, 23-25% benzaldehyde and 4-5% natural flavor. I am not able to recover E. coli according to USP <62> by using dilution as the means of neutralization. We tested it as a 1:100 dilution using TSB with polysorbate 20 and lecithin. I have read that my other options for neutralization are glycine and thiosulfate. I have not had any experience with these and would greatly appreciate any help anyone can offer.

Note: The pH at a 1:100 dilution is 7.2.

A1: At 70-75% Arabic Gum, the water activity of this material would be so low and on top of it you have 23-25% benzaldehyde. Even flavors have some antimicrobial activity depending on the vehicle used. This material in my opinion would qualify for exemption from microbial testing....unless there are issues with manufacturing, packaging and handling- in which case, no amount of testing will ensure microbial integrity since it is a powdered flavor.

A2: Benzaldehyde does have antibacterial and antioxidant activity. In general, benzaldehyde has more antimicrobial activity than benzoic acid. You do have a high concentration of benzaldehyde in your powder fruit punch favor. I do not believe that the usage of glycine or Sodium thiosulfate would be able to neutralize the antimicrobial activity of benzaldehyde.

I think the problem in your neutralization studies in obtaining the recovery of Escherichia coli is the high concentration of benzaldehyde in your fruit punch flavor. A 4% concentration of Tween in either a diluent or enrichment broth would not be able to neutralize the high concentration of benzaldehyde. Non-ionic surfactants such as Tween are able to neutralize benzoic acid. I expect that Tween will also be able to neutralize the antimicrobial activity of benzaldehyde. Because you have a 23 to 25% concentration of benzaldehyde in the fruit punch flavor, I would suggest that you make 1:1 dilution of the Fruit punch flavor into Tween 20 or 80 and mix until homogeneous for neutralization of the antimicrobial activity of benzaldehyde. From this mixture, perform a 1:10 or 1:100 plate count dilution (e.g., 2 grams into 8-ml of diluent [1:10 dilution] or 2 grams into 98-ml of diluent [1:100 dilution]).

FYI, I know that people will say that there is no need to test your fruit punch flavor for microbial content due to the high concentration of benzaldehyde. However, I disagree because your fruit punch flavor contains 70 to 74% of Arabic gum. Arabic gum is well known to be found contaminated with microorganisms.

Dry Ice blasting

Wanted to know if anyone is using dry ice blasting for cleaning of cosmetic mixers and equipment. Is cleaning effective and does it help in reducing generated waste water?

A1: I have heard that it has been used for the cleaning of food processing equipment (e.g., in bakeries). However, I have not seen anyone yet use it routinely for the cleaning of cosmetic manufacturing equipment. From what I have heard, there are some disadvantages in using dry-ice blasting for cleaning of manufacturing equipment. Because particles that are removed during dry ice cleaning are usually relatively large, these airborne particles will normally land in the immediate area in which surrounding equipment will either need to be covered or re-cleaned and sanitized. Capital investment required for dry ice blast cleaning equipment may be somewhat difficult for many small and medium sized companies to absorb. However, I do see advantages in using this technique because water or chemical cleaning agents do not need to be used for equipment cleaning.

I believe that there are some You-tube videos on using ice to clean non-sterile manufacturing equipment.

However, dry-ice blasting for equipment cleaning will need to be validated to ensure that equipment surfaces are indeed clean by using confirmatory testing such as Total Organic Carbon (TOC) analysis before equipment sanitization is performed.

Counting difficulty during suitability

My product is insoluble in Water, hence I prepared 1:10 dilution of the drug substance.

During the observation of the challenge inoculum some of the particles from my drug substance interfere with the colonies and it is difficult to count the actual colonies.

Since both the particle size and colony morphology of my challenged inoculum were similar, in some cases it resulted in false positives.

Kindly guide me to overcome this issue.

A1: You can use the MPN method or add a Redox dye to the medium to help visualize the colonies.

A2: I would request to check the specification of your product(since you didnt mentioned about the product I. e., Non sterile API, formulation).

Please find below suggestions for your query.

#1. You can dilute the product the subsequent dilution I. E., 1;50, 1:100 (check the specification of the product). By diluting the product you can overcome your below mentioned issue.

#2. I assume if your specification is <10 cfu/gram. You can use TTC dye which you can add to the media to differentiate colonies and product.

A3: TTC (2, 3, 5-Triphenyl tetrazolium chloride) is a redox indicator that can be used in microbial media to differentiate microbial cells from particles of a test sample. It is colorless in the oxidized form and is reduced to insoluble red triphenylformazan, an insoluble red pigment, by actively growing bacterial cells. The reduction of TCC to formazan is irreversible and is not re-oxidized by air once it is reduced to the red formazan. Usually 10-milliliters of a sterile 1% TTC Solution is added to 1.0-liter of microbial growth agar after sterilization.

A4: As recommended earlier, one can make in house media by adding TTC (2, 3, 5-Triphenyl tetrazolium chloride) as redox indicator) in growth media and autoclave for large scale preparation. Alternately, one could purchase prepared ready-to-use *dry-film* culture medium systems with differentiating stains for counting colonies? Use of such dry film culture media are routine in the food industry.

Media Incubation time

It is stated that "For incubation times expressed in hours, incubate for the minimum specified time, and exercise good microbiological judgement when exceeding the incubation time" in Chapter <1117>.

For pathogen test Incubation period for
MSA is 18-72 hours
CAB is 18-72 hours
XLDA is 24-48 hours
MA is 18-72 hours

My question is do we have to keep the plates for the minimum period mentioned above or within the range of time period or after the completion of incubation period in case of test of specified organisms in raw material, water, finished products etc. However for GPT we are following the minimum incubation period. Sometimes it is delayed due to holidays. What is the industry practice?

Benzyl Alcohol Endotoxin Testing

I am having trouble with endotoxin testing of pure benzyl alcohol. Testing at the MVD of 1:500 in LRW is still showing inhibition. Is there any solvents or buffers that would help overcome this inhibition?