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THE STERILITY TESTS

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BACKGROUND

The compendial Sterility Test is not a test for product sterility. This is not a new, nor a particularly insightful observation. It has been frequently presented as a flawed test for its stated purpose in the literature over the past 80 years. The test first appeared in 1932 (Brit. Pharm., 1932) and included the basic features of the modern test — two media, prescribed dilution scheme (for Bacteriostasis/ Fungistasis or method suitability) and a defined incubation time. The original test had the media incubated for five days and allowed two retests (all three had to fail to fail the test). However, the basic structure of the test was present.

This test has generated controversy as to its role in product quality testing for decades. While this is understandable, it highlights a significant problem in Quality Control (QC) pharmaceutical microbiology. We customarily use the compendial test as finished product QC release tests, but this is neither its design nor intent. Those chapters in United States Pharmacopeia (USP) numbered less than 1000 (for example, the Sterility Test is

USP chapter <71>) are referee tests — in other words they are in place solely to demonstrate conformance to qualities specified in the product monograph as described in the current National Formulary (NF) (the other part of the book). A rigid interpretation would have it that if the product is not described by NF monograph, the test does not directly apply. In fact, the preface to the internationally harmonized Sterility Tests reads:

> "The following procedures are applicable for determining whether a Pharmacopeial article purporting to be sterile complies with the requirements set forth in the individual monograph with respect to the test for sterility."

In a similar vein, sterile finished dosage forms have the following requirement in USP (from <1> *Injections*):

> "Sterility Tests — Preparations for injection meet the requirements under *Sterility Tests* <71>"

This has a nice symmetry — the test states that it is applicable for meeting the requirements set forth in the monograph, the requirement being that the material meets the requirements of the test. Note that neither USP citation requires the finished product to actually be sterile, only that it meet the requirements of the test for sterility.

So, one would have to conclude from a logical perspective that the test is not flawed for its intended purpose, that purpose being to show that the material tested meets the requirements of the test. How did we come to think that this test was designed to show the sterility of the product?

We need something to demonstrate product sterility. 21 CFR 211 states the requirement:

> "211.167 Special testing requirements.
>
> (a) For each batch of drug product purporting to be sterile and/or pyrogen-free, there shall be appropriate laboratory testing to determine conformance to such requirements. The test procedures shall be in writing and shall be followed."

The difficulty, of course, is that there really is no way, given current technology, to demonstrate sterility of a batch. This imposes significant validation issues. A way to satisfy this requirement is provided in:

> "211.194 Laboratory records.
>
> (a) Laboratory records shall include complete data derived from all tests necessary to assure compliance with established specifications and standards, including examinations and assays, as follows: ...
>
> (2) A statement of each method used in the testing of the sample. The statement shall indicate the location of data that establish that the methods used in the testing of the sample meet proper standards of accuracy and reliability as applied to the product tested. (If the method employed is in the current revision of the United States Pharmacopeia, National Formulary, AOAC INTERNATIONAL, Book of Methods,{1} or in other recognized standard references, or is detailed in an approved new drug application and the referenced method is not modified, a statement indicating the method and reference will suffice). The suitability of all testing methods used shall be verified under actual conditions of use."

So if we can cite a "validated" test we do not need to develop one ourselves. Thus the internationally harmonized Sterility Test is pressed into service as a product quality test — even though that is neither its design nor its purpose.

The compendial Sterility Test has significant limitations as a product quality test. We will discuss these limitations in the next section.

THE STERILITY TESTS

There are two different GMPs describing sterility in the United States. The first is 21 CFR 211 and the second is the "Biologics" 21 CFR 610. By common consensus, the 21 CFR 211 CGMP looks to the compendial Sterility Tests, while 21 CFR 610 describes a separate test in 21 CFR 610.12. The "Biologics" test is similar in fundamental aspects to the compendial Sterility Tests. There is a finite (and small)

sample size and two recovery media are used, each with specified incubation conditions. Both types of types (compendial and "Biologics") so share some common limitations (see below).

The compendial sterility tests describe two separate types of tests (see McGuire and Kupiec, 2007 for a recent review). In the first, solution from a specified number of containers (volume and number determined by batch size and unit fill volume) is filtered through a filter of nominal pore size 0.45 µm. Recovery of viable cells from the filter(s) is performed by submerging the filter in one of two recovery media followed by incubation at specified temperatures for 14 days. The second test is a direct immersion of the product or suspensions into a suitable volume of the two media to allow growth. The media are designed to support growth in aerobic, or growth in an environment of limited oxygen availability. This test requires demonstration that the specific method used is suitable for that product.

The US FDA Center for Biologics Evaluation and Research (CBER) version of the Sterility Test (21 CFR 610.12) has been a source of some confusion for years, as it is almost the same as (but slightly different from) the compendial test. After the years of effort put into international harmonization of the compendial Sterility Test it was hoped that CBER would just adopt it (with its flaws). However, the proposed draft (CBER, 2011) does not make this outcome seem promising. In the background material the statement is made that the USP test is acceptable as a "validated" test, but no mention of this position is made in the official text. In addition, where the current test describes the media to be used, microorganisms useful for controls, incubation temperatures and duration, and most importantly sample size, none of these are described in the proposed draft. All specific test methods have been removed to encourage the use of "validated" tests. One has to wonder what they are to be validated against if there is no official comparator. These changes will be discussed below. In any event, there is nothing in the proposed version that will prevent the use of the compendial test.

Limitations to the Sterility Tests

As early as 1956 Bryce published an article describing the two critical limitations of this test. He put forward that the test was limited in that it can only recognize organisms able to grow under the conditions of the test, and that the sample size is so restricted that it provides only a gross estimate of the state of "sterility" of the product lot (Bryce, 1956). Other concerns about the Sterility Test (e.g., choice of sample size, choice of media, time and temperature of incubation) were extensively reviewed in an article by Bowman (1969).

There have been several changes in the compendial Sterility Test since that time, culminating in the internationally harmonized test (USP, 2009). However, the two basic problems outlined in 1956 by Bryce remain today.

Sample size

The sample size is set arbitrarily, and does not provide a statistically significant population to estimate sterility (Knudsen, 1949). This is indisputable and unavoidable with a test of this type which is destructive in nature. Let's look at some of the numbers:

Let the likelihood of a contaminated unit = λ

By the Poisson distribution, the probability of picking a sterile unit from the fill (denoted P) is $e^{-\lambda}$, or $2.7182818^{-\lambda}$

Then, if you are picking 20 samples from an infinite supply (or for this discussion, from a pharmaceutical batch):

The probability of passing the sterility test is P^{20}

Conversely, the probability of failing the Sterility Test is $1 - P^{20}$

Therefore, given a known frequency of contaminated units in the batch:

Frequency of contaminated units in the batch	Probability of failing Sterility Test with the current sample size
0.001	0.0198–2%
0.005	0.0952–9.5%
0.01	0.1813–18%
0.05	0.6321–63.2%
0.1	0.8647–86.5%
0.5	1.0000–100%

The only way to change this would be to degrade the media (resulting in lesser recovery and therefore false negatives) or to increase the number of samples. Changes of this sort seem unlikely in the compendial Sterility Tests at this point in time. A discussion of different sampling plans that might be used is presented in Bryce (1956), and a fuller discussion of the controversy over the final resolution of the current procedure is provided in Bowman (1969). After extensive review, all of the proposed sampling plans were found wanting for one reason or another.

It is interesting to consider the proposed CBER Sterility Test in regards to sample size. The current 21 CFR 610.12(d)(2) states:

> "The sample used for each test medium or each incubation temperature of a test medium for the final container and first repeat final container test shall be no less than 20 final containers from each filling of each lot, selected to represent all stages of filling from the bulk vessel."

The proposed draft (21 CFR 610.12(d)) reads:

> "*The sample*. The sample must be appropriate to the material being tested, considering, at a minimum:
>
> (1) The size and volume of the final product lot;
> (2) The duration of manufacturing of the drug product;
> (3) The final container configuration and size;
> (4) The quantity or concentration of inhibitors, neutralizers, and preservatives, if present, in the tested material;"

This lack of specificity may lead to interesting audit conversations in the future.

One frequently overlooked aspect of discussions of sampling plans is that the statistical analyses all assume that the test system would recover even a single microorganism if it were present in the sample. In other words, one contaminating cell would result in media turbidity. This (unverified and unlikely) assumption leads us to the next topic.

Recovery conditions

The harmonized test utilizes Trypticase Soy Casein Digest Broth and Fluid Thioglycollate Medium. These media and their corresponding incubation temperatures were chosen to maximize recovery of potential contaminants early in the development of the tests. However, some authors have questioned the choice of media (Abdou, 1974), while others have suggested the use of solid media rather than liquid media would be appropriate (Clausen, 1973). The choices in the current harmonized procedure reflect those media to which all parties in the harmonization process could agree.

Then there was concern about incubation duration. USP 23 (1995) allowed a seven-day incubation period for products tested by membrane filtration, and 14 days for those tested by the direct transfer method. This requirement changed in USP 24 (2000) to include a 14-day incubation period for both types of tests, with the exception of products sterilized by terminal sterilization (this exception was removed by USP 27 (2004)). Similarly, the Pharm. Eur. 3rd Edition (1997) allowed a seven-day incubation period (unless mandated by local authorities). This allowance was amended in 1998 with the 4th edition to 14 days incubation. This extension was the result of concerns that the methodology might not be able to detect "slow-growing" microorganisms.

The incubation period was identified as a concern by Ernst et al. (1969) who recommended a longer period of incubation time than seven days might be necessary, perhaps as long as 30 days. More recently this position was repeated with retrospective data

provided by German and Australian workers who wished to ensure that a harmonized procedure included an incubation period of at least 14 days (Besajew, 1992; Bathgate, 1993).

However, even with the longer incubation period there is no assurance that all microorganisms can grow under these conditions, and are metabolically active. In fact a growing body of evidence suggests that there are a large number of microorganisms that are unable to replicate under standard laboratory conditions (Viable But Not Culturable — VBNC) (Rappe, 2003; Hughes, 2001; Dixon, 1998).

CLARIFICATIONS AND ENHANCEMENTS TO THE HARMONIZED STERILITY TEST

There have been quite a few clarifications offered by different regulatory agencies to the compendial Sterility Tests. This section will not be a review of the genesis of the Sterility Tests; that discussion is outside the scope of this chapter. We will, however, take a look at a few of the clarifications offered by different regulatory agencies on the implementation of the harmonized test.

US FDA/CBER

US FDA/CBER has a section of the GMP under section 21 CFR 610. In this section, 610.12 describes a separate Sterility Test to be used with those products under CBER purview. There are several differences in the test from the internationally harmonized tests that include controls, method suitability requirements, media growth promotion procedures, etc. A major difference between the tests is that the CBER test allows a retest if the original sterility test fails. This retest must also fail for the product lot to be out of specification. While the manufacturer is urged not to attempt this approach by the author of this chapter, this is still technically allowed in the Biologics sterility test. Unfortunately, the draft proposal (CBER, 2011) retains the terminology of "repeat test", but allows it only if investigation unambiguously demonstrates lab error. This is the classic definition of an invalid tes. This draft, however, does little to clarify the situation as it demands that a "repeat test" conducted on an invalid test use the same method (one assumes not the invalid one) and that

only one repeat test can be performed. This topic is discussed in more detail below.

As an aside, the pharmacopeias and 21 CFR 610.12 do not reference or provide sterility guidelines for unprocessed bulk samples for protein and virus products, although the FDA guidance documents "Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use" (FDA, 1997) and "Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals" (FDA, 1993) require this testing. Common practice is to use 10 mL/media (for a total of 20 mL) for this testing.

USP

The USP introduced clarification in 2007 with a new chapter <1208> "Sterility Testing — Validation of Isolator Systems (USP, 2007). This informational chapter provides background in isolator design and construction, the equipment qualification considerations for the isolator, validation of the decontamination cycle (this would include the internal environment, the exterior of the product containers entering for testing and the protection of the product from the decontamination cycle) and the maintenance of asepsis within the isolator environment. The reader is also instructed that a sterility test performed in a properly functioning isolator is very unlikely to result in a false positive result. Finally, instruction is provided on the training and safety aspects of the isolator operation.

Pharm. Eur.

The European Pharmacopeia has published a non-mandatory chapter "5.1.9 Guidelines for Using the Test for Sterility" (Pharm. Eur., 2009) in which further information on the Sterility Tests is provided. The user is instructed that the test can be performed in a Class A laminar air flow cabinet located in a Class B room, or an isolator. The reader is also reminded that this test cannot demonstrate sterility of a batch, and that it is the manufacturer's responsibility to adopt a representative sampling plan. Finally, elaboration is provided on "Observation and Interpretation of Results" in that during an investigation:

> "... if a manufacturer wishes to use condition (d) as the sole criterion for invalidating a sterility test, it may be necessary to employ sensitive typing techniques to demonstrate that a micro-organism isolated from the product test is identical to a micro-organism isolated from the test materials and/or the testing environment. While routine micro-biological/biochemical identification techniques can demonstrate that 2 isolates are not identical, these methods may not be sufficiently sensitive or reliable enough to provide unequivocal evidence that 2 isolates are from the same source. More sensitive tests, for example molecular typing with RNA/DNA homology, may be necessary to determine that micro-organisms are clonally related and have a common origin.'

TGA

The Australian Therapeutic Goods Administration (TGA) has published a 33-page document entitled *TGA Guidelines on Sterility Testing of Therapeutic Goods* (TGA, 2006) to explain how the harmonized Sterility Tests are to be interpreted when submitting a product into Australia, while noting that the *British Pharmacopeia* (and therefore Pharm. Eur.) is the official test. This document is extensive, and expands the details provided on controls recommended in the harmonized Sterility Test.

The Stasis Test is an additional control recommended here. In this test, spent media from a negative Sterility Test (media that have seen the membrane that filtered product and 14 days of incubation) is subjected to an additional growth promotion test to demonstrate its continuing nutritive properties.

There is also a great deal of discussion in this document on the interpretation of the test results, and on how to investigate Sterility Test failures (see below).

PIC/S

The Pharmaceutical Inspection Convention and Pharmaceutical Inspection Co-operation Scheme (jointly referred to as PIC/S) has, as its mission:

> "... to lead the international development, implementation and maintenance of harmonised Good Manufacturing Practice (GMP) standards and quality systems of inspectorates in the field of medicinal products."

There are currently 39 Participating Authorities in PIC/S (as of January, 2011 when US FDA and the Ukrainian State Inspectorate for Quality Control of Medicines were added — see *www.picsscheme.org* for current information). The organization is an important source of guidance and information (Lyda, 2007).

PI 012-2 "Recommendations on Sterility Testing"

PI 012-2 "Recommendations on Sterility Testing" provides a great deal of additional information that the inspectors are instructed to ask about. This includes direction on acceptable training of personnel, the sterility test facilities (including clean room design, airlocks, aseptic gowning and clean room fittings), cleaning and sanitization, as well as environmental monitoring of the sterility test area. Additional detail is also provided on the test method.

The Sterility Test controls are also given some attention in this document. In addition to their execution, the inspector is instructed to require a table of negative control failures and positive control failures.

The instruction provided for "validation" (or Bacteriostasis/Fungistasis) by PIC/S in this document is in conflict with the harmonized chapter. Where the harmonized chapter informs the user to add the inoculum to the final rinse, the PIC/S document states that the product should be inoculated, unless this is not practical due to product interference (such interference, presumably, would have to be documented). In addition, the PIC/S document asserts that it is good pharmaceutical practice to revalidate all products every 12 months. The author is unaware of this practice outside this document in common usage for the pharmaceutical industry. The Stasis Test is also recommended in the PIC/S document. This test is also recommended to be repeated at least every 12 months.

Finally, there is a good deal of discussion on investigations (as in the TGA guidance). This will be discussed below.

PI 014-3 "Recommendations on Sterility Testing"

This guidance document covers the same basic material as described above for USP chapter <1208> with some significant expansion on validation considerations, the nature of the sporicidal decontaminant, and the logistics of the isolator's operation. While this guidance is directed primarily to the use of isolators in manufacturing, it also claims sterility testing to be within its scope.

RMM AND THE STERILITY TESTS

A frequently discussed option for the sterility testing of finished dosage forms is to use a "rapid" method (Moldenhauer and Sutton, 2004). Currently marketed rapid microbiology methods can be grouped into two types — those that require amplification (growth) to show low-level contamination, and those that do not. In the first group would be technologies such as ATP bioluminescence, head-space analysis, and others. Examples of the second type might be technologies such as PCR and vital dye/chromatography methods. Why is this distinction important?

The concern with recovery conditions is that we do not know how to grow all microorganisms that might contaminate pharmaceutical products. Applying an alternate technology that requires growth does not result in an improvement in the sterility test method, since organisms that currently do not grow would not grow in the new method either (Moldenhauer 2006, 2010). In addition, there is the continuing concern about the duration of the incubation period.

The currently required 14-day incubation period imposes a significant burden on the manufacturer who must quarantine product until successful completion of the test. Can this be shortened in an alternate test? The time required for microbial growth to turbidity can be thought of as the sum of two stages — a lag phase

where the microorganism prepares to grow, and the generation time requirements for a low level of microorganisms to grow to a concentration where they are visible using human vision, i.e., approximately 10^7 CFU/mL. This separation of stages is important, as it seems that the lag phase is the most significant portion of time required for turbidity (Sykes, 1956). Therefore, any alternate methodology that requires growth to amplify the microorganism will likely be required to incorporate a lengthy incubation period to ensure the recovery of "slow-growing" microorganisms.

Duguid and du Moulin (2009) describe one approach to overcoming this issue. Using an amplification stage for an ATP bioluminescence technology, they started in 1999 to validate a sterility test for an autologous cell therapy product. This sterility test, which provided for product release in 72 hours with confirmatory results at the standard 14 days, was approved by FDA/CBER in 2004. In the time since, they report almost 6,000 sterility test results (samples included primary, expansion and final product from this process) were collected, including four positives detected. The alternate method detected them, on average, approximately 35 hours earlier than the confirmatory test (19 vs. 54 hours incubation). The method is a destructive one, however, leaving no material for investigations.

A group from Novartis has also pursued the ATP bioluminescence approach to an alternate sterility test with reduction of the incubation time required from 14 to five days (Gray et al., 2010). The method was based on membrane filtration, with recognition of "microcolonies" growing on the surface of the membrane placed on solid agar media. The membrane was treated with ATP-liberating reagent, which lysed the cells, and then bioluminescence reagents to identify the microcolonies by light emission. This same group claims great success in "regrowth" of these colonies by transferring the treated membrane to fresh media for incubation, allowing subsequent identification of the contaminants (Gray et al., 2011).

The US FDA/CBER (the "Biologics" group) has issued a draft guidance document on the validation of growth-based rapid methods for use in sterility testing (FDA 2008). This CBER

document is remarkable in its complete avoidance of any mention or consideration of the previous work done in validation of RMM by FDA/CDER, Pharm. Eur., USP or PDA. This is supported and continued in the CBER draft sterility test proposal (CBER, 2011) which admittedly makes it simpler to have an alternate sterility test by removing any specifics (microorganisms, media, incubation duration, incubation temperatures, sample size/plan, etc.) from the test, and including reference to non-growth based methods.

The limiting aspects of growth-based methods as an alternative for the sterility test can be avoided by use of a Rapid Microbiological Method (RMM) technique that does not require growth (Moldenhauer, 2006). The use of a method that avoids growth requirements offers an additional advantage in that the question of VBNC organisms is completely side-stepped. As no culturing is required, the recovery phase of the Sterility Tests can be optimized to all microorganisms, regardless of growth requirements. This approach is described by Gresset et al. (2008) using a vital dye. Their discussion was expanded (Smith et al., 2010) with evaluation of different approaches to regulatory acceptance of alternate sterility tests for non-growth based test methods. The difficulty with this approach is that the viability of the treated cells is uncertain, raising questions about the ability of the test to submit to investigation.

A second promising vital-dye based approach is through the use of capillary electrophoresis of stained samples. While this method has been shown to be feasible (Bao, 2010) the sample size involved is very small (less than 1 mL), and this may pose problems for its use in QC release testing.

INVESTIGATIONS IN THE STERILITY TEST

There is a significant amount of literature written on out-of-specification (OOS) and investigations. Most of this concern, of course, stems from the 1993 Barr Decision (Madsen, 1994). Barr Laboratories had a history of repeated current good manufacturing practice (cGMP) deficiencies, including repeated retesting and resampling of product as well as reprocessing of defective product without adequate justification in a practice that has come to be

known as "testing to compliance." This is not good practice — the OOS data are telling the manufacturer important information about the product and must be resolved. Unfortunately for the microbiology community, this initial situation, as well as most of the subsequent writing on this topic, has focused on OOS from an analytical chemistry perspective. The Food and Drug Administration (FDA) has provided guidance following the *Barr* decision, and drafted the "Guidance for Industry — Investigating Out of Specification (OOS) Test Results for Pharmaceutical Production" (FDA, 2006). Interestingly, this guidance document only briefly touches upon microbiological data, stating that "the USP prefers the use of averages because of the innate variability of the biological test system." In addition, this guidance document specifically excludes microbiology from its scope in footnote 3.

A PDA task force looked into this issue and suggested the use of the phrase "Microbial Data Deviation" (MDD) in the investigation of issues in microbiology, at least until it is clear that the issue is a true product specification failure, as opposed to a lab error or process monitoring concern (reviewed in Sutton, 2007). This terminology has been adopted by USP (USP, 2010).

The harmonized Sterility Tests provide some guidance on investigations:

> "If evidence of microbial growth is found, the product to be examined does not comply with the test for sterility, unless it can be clearly demonstrated that the test was invalid for causes unrelated to the product to be examined. The test may be considered invalid only if one or more of the following conditions are fulfilled:
>
> a. The data of the microbiological monitoring of the sterility testing facility show a fault.
>
> b. A review of the testing procedure used during the test in question reveals a fault.
>
> c. Microbial growth is found in the negative controls.
>
> d. After determination of the identity of the microorganisms isolated from the test, the growth of this species (or these species) may be ascribed unequivocally to faults with respect to the material and or the technique used in conducting the sterility test procedure.

> If the test is declared to be invalid, it is repeated with the same number of units as in the original test. If no evidence of microbial growth is found in the repeat test, the product examined complies with the test for sterility."

Conditions (a) and (b) basically refer to a catastrophic failure of control. If it can be demonstrated that either the technique or the environment was not in control at the time of the test, the test can be declared invalid.

Condition (c) is interesting in its own right. The assumption when running a control is that the effort to run that control is justified by the information provided by the test. However, it seems common practice to only consider the results from the negative control if the test fails. In other words, although the negative control is supposed to demonstrate the adequacy of the test conditions and performance, if the test samples pass, then a failing negative control is ignored. If the test samples fail, a failing negative control is used to invalidate the test. The author of this chapter urges that a consistent interpretation of controls be used.

Condition (d) is one that has received a great deal of attention. Additional detail is provided in the previously cited Pharm. Eur. 5.1.6, the PIC/S guidance on sterility test and the TGA document. This topic is also discussed in FDA's Aseptic Manufacturing Guide (FDA, 2004). Reduced to its essentials, the user is urged in these documents to use methods sensitive enough to demonstrate that the microorganism is not only of the same species, but also of the same strain or sub-strain of that species. It should be noted that even with this detail the best that can be done is to show a correlation between the presence of the strain from the two sources, rather than a causal relationship. In other words, finding the same strain of *Staphylococcus aureus* on the testing technician and in the sterility test does not prove that the only possible source of the microorganism was the technician (the strain could also be present in the aseptic core), but it is accepted as sufficient proof in regulatory guidance that the test was compromised and so invalid.

We need to spend a moment on the concept of "invalid" with regards the Sterility Test. Original testing conditions in the

microbiology lab were not stringently aseptic, and false positive results were not uncommon. In recognition of this fact, the compendial tests allowed a "second tier" sterility test which used twice the number of samples and was to be conducted if the first test failed. Passing the second tier test was taken as evidence that the sample met the requirements of the test. In the years after the *Barr* decision, and with improvements in in test methods, this practice was removed from the compendial Sterility Tests as inappropriate. In its place was provided detailed discussion of how to investigate, and perhaps invalidate, a sterility test result. By definition, an invalid test is not valid and the lab is expected to perform the test in a valid manner.

The "repeat test" does, however, remain in the CBER Sterility Test (21 CFR 610.12(b)). The draft CBER test does not clarify this greatly, retaining the terminology "repeat test" while requiring an investigation to invalidate the original test. The proposed text then requires the same method be used in the repeat test, as was used in the invalid test, and allowing only one "repeat test" per lot.

Investigations of sterility tests are obviously a source of continuing controversy. The pharmaceutical literature provides some examples of Sterility Test investigations that can be used as guides. Lee (1990) described a detailed sterility investigation that included the identification of the contaminant, reviews of documents, training records, gowning practices, environmental monitoring records, lab procedures and other critical controls. It should be stressed here that most of the work in an investigation occurs reviewing records. The practice of complete proactive documentation is critical to the success of any investigation. The likelihood of an inconclusive investigation (and therefore surety of failing product) is assured if the associated records do not support a definitive finding.

Schroeder (2005) published a thoughtful review of considerations for a sterility failure investigation. He argues that for products sterilized by filtration that filter failure must also be considered in addition to the other commonly cited areas of investigation.

CONCLUSIONS

The current harmonized Sterility Test has two fundamental weaknesses, both of which have been obvious from its inception. The first is that the sampling plan is insufficient to meet the requirements implied by the title of the test. This weakness is not solvable in the current regulatory climate (nor has it been for over 70 years). The second weakness of the test involves recovery and recognition of microbial contamination in the sample, should it exist. There are several different varieties of the Sterility Test, and even when citing the harmonized test the user must be sensitive to regional expectations for that test. While there is great promise in finding a rapid method for conducting sterility tests, few examples exist of this having been successfully accomplished. Finally, there are clear expectations on the investigations to conduct into a failed Sterility Test, and the user is urged to be familiar with these expectations.

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