Medical Device Sterility Testing —

Industrial sterility and the USP sterility test method explained: Are my products sterile?

Abstract:

Sterility testing of medical devices is required during the sterilization validation process as well for routine quality control. ISO standards for both gamma and electron beam sterilization employ sterility testing as a measure of adequacy of sterilization parameters. An understanding of sterility testing is beneficial in terms of designing a validation process. The need to provide adequate and reliable sterility test data is an important quality-control issue. Sterility testing is a very tedious and artful process that must be performed by trained and qualified laboratory personnel. The investigation of sterility test failures is a process that requires attention to environmental data as well as many other factors including training and sample difficulty. When these processes are performed properly, sterility testing can be a regulatory and legal benefit during any potential recall activities.

This paper presents the general concepts and problems associated with sterility testing as well as the various testing methodologies. Most USP<71> sections are harmonized with the EP/JP. However, there are other regulators that may require additional incubation times or temperatures. Microorganisms can grow at very diverse temperatures. These temperatures can be added to the analytical method during the validation phase for each product. Current USP sterility tests take 14 days to complete. This is too long for compounding pharmacies, cellular therapy, and PET injections to meet patient treatment schedules. Currently, rapid sterility methods can be validated under Section <1223> Validation of Alternative Microbiological Methods. Some rapid methods can detect viable microorganisms in the 1-3 CFU level. USP has convened an expert panel to recommend changes to the USP that add rapid methods as part of the referee testing.

Introduction

Medical device sterility testing is an essential part of every sterilization validation program. Sterility testing is an extremely difficult process that must be designed to eliminate false-positive results. False-positive results are generally due to laboratory contamination from the testing environment or technician error. The environment must be designed to meet the requirements of the United States Pharmacopeial (USP)[2] in terms of viable microbial air and surface counts. Growth media used in sterility testing must be meticulously prepared and tested to ensure its ability to support microbial growth. The most difficult to sterilize area(s) should be defined for each medical device. Procedures for sampling, testing, and follow-up must be defined in the validation procedure.

Sampling Plans

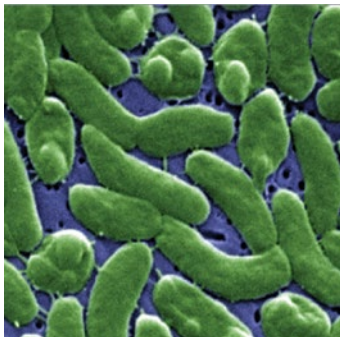
The official test, the USP (volume 38: 2015) requires 40 units per production lot. In cases where small lots (less than 1000 devices) are manufactured, the sampling depends on the lot size (see USP sampling plan matrix). The ISO documents for radiation sterilization and ethylene oxide sterilization, ISO 11137/11135, outlines the requirements for sampling during validation activities.

Sampling sizes for small clinical lots or first release of small batches can be accomplished using the ISO methods (ISO 11135 Annex E). Ethylene oxide sterilization validation requires product and biological indicator sterility testing. The validation should contain sufficient product test units and biological indicators that meet the requirements of the 11135 document. Additional white papers will cover both ethylene oxide and radiation sterilization validation requirements.

Resistant Microorganisms: Past and Future — Risk Analysis

Radiation sterilization validation following the ISO 11137 document requires dose-setting sterility testing that determines if the device bioburden population is less resistant to radiation sterilization than historical medical device bioburden data. Some organisms are resistant to radiation and ethylene-oxide sterilization. *Deinococcus radiodurans* and *Pyronema domesticum* (a fungus) are extremely resistant to ionizing-radiation sterilization. These organisms can be cause for recalls and should be assayed for during any sterilization validation process. *Pyronema domesticum* was the cause for a major cotton products recall in 1993 (Med-line industries) This organism is extremely resistant to both radiation and ethylene oxide sterilization (ref). Emerging microorganisms, such as *Vibrio vulnificus* (photo 1), which is a flesh eating bacteria species found in sea water and shellfish, and *Staphylococcus aureus,* which can cause infections and death, have the potential to obtain radiation or ethylene oxide gas resistance. Work has shown that these genes can be transferred to other species by natural selection and plasmid transfer. Like *Pyronema domesticum*, these organisms may not show up in USP sterility testing. *Vibrio* requires complex growth media (1% NaCl) and would not grow out in the USP/ ISO sterility testing media. *Staphylococcus aureus* (if damaged by sterilizing processes) may take more than the 14 days required under the USP sterility test to grow and show turbidity. Some organisms (*Listeria*, P *Thermoacidurans*, *Geobacillus stearothermophilis)* and other thermophiles could be contaminating medical devices without being detected by the current methods. Water-loving bacteria (Pseudomonas) found in water lines and process water systems are extremely difficult to culture. These organisms would not be found during a standard bioburden test. Therefore, it is extremely important that your release program is supported with additional data that gives one a microbial detection level.

Photo 1



Risk analysis of most medical devices from these organisms is low. However, quality systems programs should entertain looking past the USP sterility tests and look for any viable microorganisms including viruses and parasites. We assume that since there is very little literature regarding viral contamination, that it would never happen. Mycoplasma, viral, and protozoan contamination can be a concern with products that have a cell-culture component. Therefore, the USP sterility test should not be the sole measure of sterility. It is a legal test that the FDA uses to determine the sterility of a lot.

Environmental Concerns Related to Sterility Testing

The sterility test environment is described in USP General Informational Chapter <1211>. The environment should be as stringently controlled as an aseptic processing environment. The FDA published in September 2004 industry guidance entitled: *Sterile drug products produced by aseptic processing — Good Manufacturing Practice*. This aseptic processing guideline outlines the requirements for manufacturing sterile pharmaceuticals. An aseptic

processing environment (clean room) is used to dispense sterile pharmaceuticals into presterilized containers. A clean room is generally a room that delivers laminar flow air that has been filtered through microbial-retentive high-efficiency particulate air (HEPA) filters. The room is maintained under positive pressure and has specifications for room air changes per hour. An environment used for sterility testing should be similar in design to an aseptic processing environment: there must be an anteroom for gowning and separate area for the actual sterility testing. The testing area should meet ISO Grade 5 (grade A) particulate control requirements (specified in USP chapter <1116>). Sterility testing should not be carried out under a laminar flow hood located within a room that is not maintained as ISO Class 5. Along with particulate testing in the environment, the laboratory must test for viable bacterial and fungal organisms ubiquitous to it. The sterility test technician must be suitably gowned in sterile garments that prevent microbial shedding into the room and contaminating the product under test. The room must be validated in terms of particulate and microbial levels (ref). Microbial levels must be routinely monitored for sterilization process-type resistant microorganisms. All microorganisms found must be identified as per genus and species. Genetic-type microbial identification systems are an excellent way to positively identify these environmental contaminates. Environmental contaminates (Bacillus sp., P. acnes, Micrococcus, Staphyloccocus sp.) are problematic for all medical device manufacturing processes.

The laboratory must have a validation and training program for gowning and sterility testing. Our validation program requires that technicians consecutively test 40 simulated samples for both membrane filtration and direct immersion methods without a false-positive test result under less ideal environmental conditions (worst case).

Methodologies

The USP is a compilation of validated methods and official monographs for pharmaceutical and medical devices. The USP is divided into the following sections: Monographs, General Informational Chapters, and General Requirements. General Informational Chapters are not legal requirements. The sterility test (USP<71>) is categorized under General Requirements. It is therefore a legal requirement.

The ISO radiation sterilization microbial methods (11737-2 1998)[4] describes a sterility test which is a modification for the USP method. This test is specific for the detection of mesophilic aerobic organisms that have been exposed to sub-lethal sterilization cycles. This test will not detect anerobics, thermophilics, thermophilic deinococcus, psychrotrophics, and many fungi species. Psychrophilic microorganisms have a maximum temperature for growth at 20°C or below and are restricted to permanently cold habitats. This ISO sterility test method is recommended for the validation of both gamma- and electron-beam sterilization cycles. ISO recommends that the sterility test be validated by using know sterile products.

The method of choice for EO 5 sterilized product is the official USP <71> procedure using both SCDM (aerobic) and FTM (anaerobic) media.

Harmonization

Portions of this general chapter 71 have been harmonized with the corresponding texts of the European Pharmacopeia and/or the Japanese Pharmacopeia. Those portions that are not harmonized are marked with symbols (✦✦) to specify this fact.✦. The laboratory will instruct you when there is a conflict with harmonization. Generally, it has to do with media, bacteria, or medical device testing.

Processes

Prior to actual sterility testing, it is prudent to send an example sample to the testing laboratory so the laboratory personnel can determine the appropriate testing procedure. Each product should have a unique procedural specification for testing (Client SOP). This procedure should be very specific in terms of which items to test (in the case of kits), and indicate the Sample Item Portion (SIP). The SIP is the percentage of the complete tested. Medical devices come in all shapes and sizes. For large and cumbersome devices, it is very difficult to test them in their entirety. Therefore, the test laboratory will determine a SIP that is a portion of the sample expressed in fractional terms (i.e. 0.1 for 10 percent of the sample). This number is used for ionizing radiation sterilization dose-setting methods. The SIP portion should be validated by a suitability test to determine if the bioburden is homogeneous throughout the device or kit. Sometimes the SIP should be based on bioburden SIP and not weight or volume SIP. Choosing the correct SIP is critical for the validation. (A future white paper explaining ionizing radiation sterilization (ISO 11137) and EO sterilization nuances is planned. Please check back.)

Combination products have unique challenges. A combination medical device product is defined as one that has a drug or biologic component integrated with a medical device — for example, a drug coated stent. The agency's Office of Combination Products would determine which regulatory branch (CDRH,CDER or CBER) is officiating the product. Official USP sterility testing of combination products is required for all sterile drug and biologics products. The drug product component applied aseptically creates the largest challenge to the manufacturer. For example, albumin-coated vascular grafts are aseptically manufactured and then terminally sterilized. Microorganisms grow very rapidly. They can overload the sterilization dose that reduces the probability of a non-sterile or sterility assurance level (SAL) from 10-6 to something less than what is considered sterile from a regulatory standpoint. Industrial sterilization is not an absolute process. It is a probability of a non-sterile device based on environmental and bioburden data. A change in bioburden data will result in a change in SAL.

Biologics must be aseptically processed and cannot be terminally sterilized. We are seeing more biologics being developed that have a medical device delivery component. Combination products sterilized by radiation are generally handled as medical devices following ISO 11137 dose-setting methods. For the most part, pharmaceutical GMPs would take precedent over 21 CFR section 820 Quality System Requirements with all combination products. The more robust GMP requirements would assure reduced bioburden counts and consistent microbial populations during manufacturing.

The USP<71> Sterility Test contains two qualifying assays that must be performed prior to sterility testing. They are the "Suitability Test" (Growth promotion Test) and the "Validation Test" (Bacteriostasis and Fungistasis Test).

The Suitability Test is used to confirm that each lot of growth media used in the sterility test procedure will support the growth of less than 100 viable microorganisms. If the media cannot support the growth of the indicator organisms, then the test fails. Secondly, a portion of each media lot must be incubated and assessed for sterility according to the incubation parameters (time , temperature) established by the method. If the media is found to be non- sterile, then the test fails.

The Validation Test is used to determine if the test sample will inhibit the growth of microorganisms in the test media. Stasis, in terms of microbiology, is defined as the inability of a microorganism to grow and proliferate in microbiological media. Media that is bacteriostatic does not necessarily kill bacteria; it simply may retard bacterial growth and proliferation. The Validation Test must be performed on each product prior and/or during sterility testing (ref. FDA Pharma Micro Manual[6]). This test determines if the media volumes are valid for the particular product. Some medical devices contain bacteriostatic and fungistatic compounds that may require special

procedures and special media for testing. This test is similar to the Suitability Test described above. However, the product sample is placed in the media along with the indicator microorganisms. Microbial growth in the presence of the test samples is compared to the controls without test samples. If microbial growth is present in the sample and control containers, then the test is valid. The next step is to proceed to actual sterility testing. Suitability, Validation, and Sterility tests can be performed simultaneously.

The USP describes three general methods for sterility testing: 1) Membrane filtration; 2) Direct transfer (product immersion); and 3) Product flush.

Membrane Filtration Sterility Testing

The membrane filtration sterility test is the method of choice for pharmaceutical products. **It is not the method of choice for the majority of medical devices**. The FDA may question the rationale behind using the membrane filtration test over the direct-transfer test for devices. Some medical devices are liquid such as transfusion fluid used for organ maintenance prior to transplantation. An appropriate use of membrane filtration technique is for devices that contain a preservative and are bacteriostatic and /or fungistatic under direct-transfer methods. Povidone iodine (Betadine™) and 70% IPA wipes are such devices. With membrane filtration, the concept is that microorganisms will collect on the 0.45 micron filter surface. This pore size is selected because the preponderance of microorganisms will be collected on the surface. It is not a sterilizing grade filter. Some microorganisms are small enough to pass through this size filter: *Brevundimonas diminuta, Mycoplasma*, and viruses. We use this filter because it will not clog with large volumes of fluid. However, it will not collect all microorganisms. Some processed water bacteria are very small due to their low nutrient environment. These microorganisms would be difficult to culture and would not grow in the USP media. After filtering the sample, the filter is segmented and transferred to appropriate media. The test media are fluid thioglycollate medium (FTM) and soybean casein digest media (SCDM). FTM is selected based on its ability to support a wide variety of both anaerobic and aerobic microorganisms. SCDM is selected based on its ability to support a wide range of aerobic, bacteria, and fungi (i.e. yeasts and molds). The incubation time is 14 (check) days. Since there are many manipulations required for membrane filtration medical device sterility testing, the propensity for laboratory contamination is high. Therefore, in an open system, more sterility failures are expected when using this method. A closed system is recommended for small devices or combination products. Using commercially available sterility testing kits are the most robust way to reduce potentially false positives. These kits are pre-sterilized and are closed systems that require very little manipulation.

Direct-Transfer Sterility Testing

This is the method of choice for medical devices because the device is in direct contact with test media throughout the incubation period. Viable microorganisms that may be in or on a product after faulty/inadequate sterilization have an ideal environment within which to grow and proliferate. This is especially true with damaged microorganisms where the damage is due to a sub-lethal sterilization process. All microorganisms have biological repair mechanisms that can take advantage of certain environmental conditions conducive for repair and proliferation. The direct-transfer method benefits these damaged microorganisms. The entire product should be immersed in test fluid. With large devices, patient contact areas should be immersed. Large catheters can be syringe filled with test media prior to immersion. Cutting catheter samples to allow for immersion is the method of choice.

The USP authors understand that appropriate modifications are required due to the size and shape of the test articles. The method requires that the product be transferred to separate containers of both FTM and SCDM. The product is aseptically cut, or transferred whole, into the media containers. The test article should be completely immersed in the test media. The USP limits the volume to 2500 mL. After transfer, the samples are incubated for 14 days.

Product Flush Sterility Testing

The product flush sterility test is reserved for products that have hollow tubes, such as transfusion and infusion assemblies, where immersion is impractical and where the fluid pathway is labeled sterile. Our lab does not see many devices with just the pathway labeled sterile. This method is relatively easy to perform and requires a modification of the FTM media for small lumen devices. The products are flushed with fluid D, the eluate is membrane filtered, and the filter is placed into FTM and SCDM media. This method is not generally used.

Interpretation of Sterility Test Results

The technicians must be trained as to how to detect growth during the incubation period. Growth is determined by viewing the media, which is generally clear and transparent, against a light source. Turbidity (cloudy) areas in the media are indicative of microbial growth. Once growth is detected, the suspect vessel is tested to confirm that the turbidity present is due to microorganisms and not due to disintegration of the sample. Sometimes samples produce turbidity because of particulate shedding or chemical reactions with the media. Once a suspect container has been tested, it should be returned to the incubator for the remainder of the incubation period. Samples that render the media turbid are transferred on Day 14 of the test and incubated an additional 4 days. Growth positive samples require further processing such as identification and storage.

Sterility Test Failure Investigation

For every positive sterility test or out of specification test (OOS), the laboratory should perform an OOS investigation to determine the validity of the positive growth from the medical device sample. The investigation encompasses the following items: 1) clean room environmental test excursion review (EER); 2) media sterilization records; 3) technician training records; 4) the relative difficulty of the analytical procedure; 5) control data (open and closed media controls); 6) technician sampling data (microbial counts on gloves and /or garments post testing).

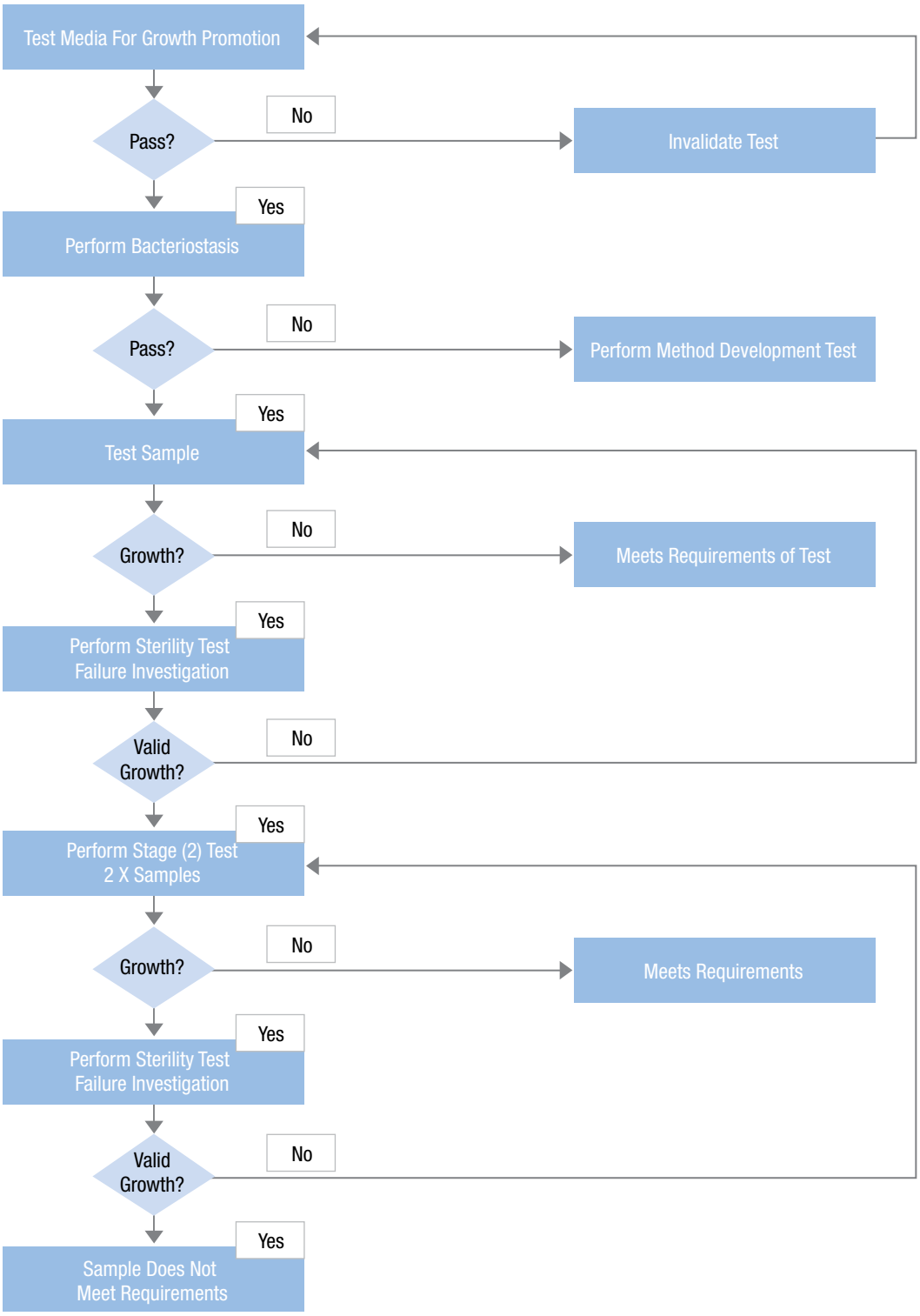
The USP allows for a retest of the products if persuasive evidence exists that the cause of the initial sterility failure was induced by the laboratory. Identification and speciation of the isolate(s) are significant contributing factors to the final decision. Nothing found is insignificant. Second-stage sterility test can be invalidated by the laboratory, and then the USP allows for second-stage sterility testing. Second-stage sterility testing requires double the original number of samples tested. The second-stage test can be repeated if evidence exists invalidating the test due to laboratory error as above.

A detailed investigation may uncover circumstantial evidence to support a final decision. It is recommended that sterilization cycle data, environmental data, and bioburden data be reviewed prior to making any decision to release the product.

It is recommended that medical device manufacturers qualify the test procedure with inoculated samples. These inoculated samples should be to determine the hardest to sterilize areas such as syringe plungers and check valves.

The probability of a false positive can be calculated using John Lee's formula.[7] The formula is based upon sample container diameter, amount of time the container is left open, and the ISO 5 particulate level.

USP Sterility Testing



USP Sterility Testing Sampling Plan Matrix

USP Official Requirement

Batch Size	Sample Size
Normal Lots > 1000	40 Articles
< 100	0% or 8 (whichever is greater)
100-500	20 Articles
500-1000	4% of 40 (whichever is less)

Sterilization ISO Standards

ISO 11135	Ethylene Oxide Sterilization (Biological Indicator Qualification Study) Follow USP sterility method 40 product sterility
ISO 11137	Radiation Sterilization Procedure Method 1: 100 VDA 100 SDA Single Batch Sterility Test 100 Samples VDA (only) VD_{Max}: 25 kGy Method 10 VDA and CVDA Single and Multiple Batch Test
ISO 15843	Alternate Sampling Schemes for 11137 A: 52 VDA 50/70/140 SDA B: 60 VDA 35 SDA

VDA – Verification Dose Audit
SDA – Sterility Dose Audit
SCDM – Soybean Casein Digest Media USP
FTM – Fluid Thio Glycollate Media USP
CVDA – Conformatory Dose Exp. VDMax

USP Sterility Testing

Methods

1. Membrane Filtration
   First choice for pharmaceutical products or medical devices with Bacteriostatic/Fungistatic properties
   14 Day Incubation
2. Direct Transfer (Product Immersion)
   Method of choice for medical devices
   Complete immersion recommended: 2500 mL Max. Volume
   14 Day Incubation
3. Product Flush

Recommended for transfusion and infusion assemblies that indicate a sterile fluid pathway that cannot be cut without contamination sample.

What do you do when a sterility test is positive?

Possible rationale for false positives: Contaminated outer pouch prior to sterility test. Failures can be attributed to gross outer package contamination. Very difficult to disinfect the outer packaging. Solutions?

Laboratory error due to extrinsic contamination: Solutions?

Analyst contamination: Solutions?

Difficult Sample: I.E. takes 10 minutes to cut it and place it into a test vessel.

Sterility Testing Environmental Issues

1. The USP indicates that a 10-3 level of non-product contamination is required. This is similar to the efficiency of an aseptic processing area and comparable to the microbial efficiency of aseptically processed pharmaceutical. This relates to one non-sterile out of 1000 processed units.

   A validation of the sterility suite is recommended. (See MDM article SGR)
2. Gowning validations.
3. Identification of bacterial and fungal isolates. (Micro seq™)
4. Cleaning and disinfectant validations.
5. Trend analysis of false positives: FDA guideline requires less than 0.5% false positive rate.
6. Frequency of air/surface sampling in the sterility suite.
7. Certification of the room as ISO Class 5 or better.
9. Validation of garment sterilization.
10. Action and alert levels for surface and air viable contaminates.

Investigating Sterility Test Failures (OOS)

Sterility retests are valid only if "persuasive evidence" exists to show that the cause of the initial sterility test failure was induced in the laboratory.[7]

1. Identification and speciation of the isolate is a significant contributing factor to the final decision of an action plan.[7]
2. Review pertinent records
   a. Review component sterilization data
   b. Review environmental monitoring data
   c. Trends in sterility tests
   d. Review Bioburden data
   e. Laboratory procedures
   f. Review of package integrity
3. Employee practices
4. Equipment and Components
   a. Validated cycle parameters
   b. Equipment malfunction
   c. Manufacturing processes
5. Laboratory Investigation
   a. Environmental testing (IDs)
   b. Media sterilization
   c. Training
   d. Sample procedure (level of difficulty)
   e. Controls

Conclusion

Sterility testing requires high levels of control with regards to CFR Quality Systems Requirements[8], Good Laboratory Practices[9], environment (clean room ISO Class 5 or better), and employee practices. It is essential that meticulous technique be employed. Sterility testing is an integral part of sterilization validation as well as routine quality control. As I mentioned, there are numerous organisms that will not grow and proliferate using the USP method. These organisms should be found during bioburden testing and eliminated. Risk analysis for the sterilization validation processes is high. The modulators of the risk are mentioned above. A detailed investigation may uncover circumstantial evidence to support a final decision. Follow-up investigations before making any final decisions for product release have been recommended.[7]

References

1 ISO 11137, Sterilization of health care products

2 The United States Pharmacopeia, 38th revision, The United States Pharmacopeia Convention: First Supplement: August 1, 2015

3 USP 38 Table 3: Minimum Number of Articles to be Tested in Relation to the Number of Articles in the Batch

4 ISO 11737, ANSI/AAMI/ISO 11737-2 1998, Sterilization of Medical Devices — Microbiological methods — Part 2, Tests for sterility performed in the validation of a sterilization process

5 ISO 11135 1994, Medical Devices Validation and Routine Control of Ethylene Oxide Sterilization

6 FDA Pharmaceutical Microbiological Manual: June 2015: 2015-06-26 | www.fda.gov/downloads/scienceresearch/fieldscience/ucm397228.pdf

7 Lee, John Y., "Investigating Sterility Test Failures," *Pharmaceutical Technology*, February 1990

8 Code of Federal Regulations, Title 21/Chapter I/Part 820, "Quality Systems Requirements: General," 2015

9 Code of Federal Regulations, Title 21/Chapter I/ Part 58, "Good Laboratory Practice for Nonclinical Laboratory Studies," 2015