STERILITY TESTING – ESSENTIAL THINGS YOU MUST KNOW

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(includes combination products)

**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 the 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.

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.

**INTRODUCTION**
Medical device sterility testing is an essential part of every sterilization validation. Sterility testing is an extremely difficult process that must be designed so as 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) 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 procedures.

**SAMPLING PLANS**
The official test, the USP (Volume 29) recommends testing 40 units per production lot. In cases where small lots (>1000) are manufactured, the sampling size depends on lot size (see chart).
The ISO 11137/11135 standards recommend various sterilization validation sampling plans based on lot size and validation method.

**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. 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 which has been filtered through microbial retentive High Efficiency Particulate Air (H.E.P.A.) 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 should be an anteroom for gowning and a separate area for the actual sterility testing. The testing area should meet ISO Class 5 particulate control requirements (specified in USP 1116 chapter).
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. The room should be validated in terms of particulate and microbial levels.
The laboratory must have a validation and training program for gowning and sterility testing. Our validation programs require that technicians consecutively test 40 simulated samples for both membrane filtration and direct immersion methods without a false positive test result under less than ideal environmental conditions.

**METHODOLOGIES**

The United States Pharmacopeia is a compilation of validated methods and official monographs for pharmaceuticals and medical devices. The USP is broken down into the following sections: Monographs, General Informational Chapters, and General Requirements. General Informational Chapters are not legal requirements. The Sterility Test (USP Section <71>) is categorized under General Requirements and is therefore a legal requirement.

The ISO radiation sterilization microbial methods (11737-2 1998) describes a sterility test which is a modification for the USP method. This test is specific for the detection of aerobic organisms which have been exposed to sub-lethal sterilization cycles. This ISO sterility test method is recommended for the validation of both gamma and electron beam sterilization processes. ISO recommends that the sterility test be validated by using known sterile products.

The method of choice for EO sterilized products is the official USP <71> procedure.

**PROCESSES**

Prior to actual sterility testing, it is prudent to send an example sample to the testing laboratory so the laboratory can determine the appropriate testing procedure. Each product should have a unique procedural specification for testing. The 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 product 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 which is a portion of the sample expressed in fractional terms (i.e. 0.1 for 10 percent of the sample). This number is used in gamma and electron beam dose setting methods. The SIP portion should be validated by sterility testing. (A future white paper explaining radiation and EO validation methods is planned. Please check back.)

Combination products have unique challenges. A combination product is defined as one that has a drug component integrated with a medical device. For example, a drug coated stent. The agency 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 products. The drug product component applied aseptically creates the largest challenge to laboratory personnel. Biologics must be aseptically processed and cannot be terminally sterilized. In the short future, we will see more biologics that are combination products. Combination products sterilized by radiation are generally handled as medical devices following the ISO 11137 standard. For the most part, pharmaceutical GMPs would take precedent over 820 QSR requirements with all combination products. The more robust GMP requirement would assure reduced bioburden counts and consistant microbial populations during manufacturing.

The USP <71> Sterility Test contains two qualifying assays which 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. This test determines if the media volumes are valid for the particular product. Some medical products 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 microorganisms. Microbial growth in the presence of the test samples is compared to 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 medical devices; the FDA may question the rationale behind using the membrane filtration test over the direct transfer test for devices. An appropriate use of this test is for devices that contain a preservative and are bacteriostatic and/or fungistatic under the direct transfer method. With membrane filtration, the concept is that the microorganisms will collect on the surface of a 0.45 micron pore size filter. This filter is segmented and transferred to appropriate media. The test media are fluid thioglycollate medium (FTM) and soybean casein digest medium (SCDM). FTM is selected based upon its ability to support the growth of anaerobic and aerobic microorganisms. SCDM is selected based upon its ability to support a wide range of aerobic bacteria and fungi (i.e. yeasts and molds). The incubation time is 14 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.

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 environmental conditions conducive to growth. 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 complete immersion is the method of choice.

The USP authors understand that appropriate modifications are required due to the size and shape of the test samples. 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 media volume to 2500 mL. After transferring, 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 as sterile. This method is easy to perform and requires a modification of the FTM media for small lumen devices. The products are flushed with fluid D and the eluate is membrane filtered and placed into FTM and SCDM. This method is not generally used.
INTERPRETATION OF STERILITY TEST RESULTS

The technician 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. Turbid (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 for 4 days. Growth positive samples require further processing such as identification and storage.

STERILITY TEST FAILURE INVESTIGATION

For every positive sterility test (OOS), the laboratory should perform an OOS investigation to determine the validity of the positive growth. This investigation encompasses the following items: 1) clean room environmental test (EER) data; 2) media sterilization records; 3) technician training records; 4) the relative difficulty of the test 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 product if persuasive evidence exists to show that the cause of the initial sterility failure was induced by the laboratory. Identification and speciation of the isolate(s) is a significant contributing factor to the final decision. If the First Stage sterility test cannot be invalidated by the laboratory, 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 a 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 product.

It is recommended that medical device manufacturers qualify the test procedure with non-sterile samples. The probability of a false positive can be calculated using John Lee’s formula. The formula is based upon sample container diameter, amount of time the container is left open, and the room particulate count.

CONCLUSION

Sterility testing requires high levels of control with regards to CFR Quality Systems Requirements, Good Laboratory Practices, environment (aseptic 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 a routine quality control. False positive results are common and should be planned for.

REFERENCES

3. USP 29 Table 3: Minimum Number of Articles to be Tested in Relation to the Number of Articles in the Batch
5. ISO 11135 1994 Medical Devices Validation and Routine Control of Ethylene Oxide Sterilization
8. Code of Federal Regulations Title 21/Chapter I/Part 58, “Good Laboratory Practice for Nonclinical Laboratory Studies,” 2006
USP Sterility Testing

Test Media For Growth Promotion

- Pass?
  - Yes: Perform Bacteriostasis
  - No: Inactivate Test

Perform Bacteriostasis

- Pass?
  - Yes: Test Sample
  - No: Perform Method Development Test

Test Sample

- Growth?
  - No: Meets Requirements of Test
  - Yes: Perform Sterility Test Failure Investigation

Perform Sterility Test Failure Investigation

- Valid Growth?
  - No: Perform Stage (2) Test 2 X Samples
  - Yes: Meets Requirements

Perform Stage (2) Test 2 X Samples

- Growth?
  - No: Meets Requirements
  - Yes: Perform Sterility Test Failure Investigation

Perform Sterility Test Failure Investigation

- Valid Growth?
  - No: Sample Does Not Meet Requirements
  - Yes: Meets Requirements
### Sterility Testing Sampling Plan Matrix

#### USP Official Requirement

<table>
<thead>
<tr>
<th>Batch Size</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Lots &gt; 1000</td>
<td>40 Articles</td>
</tr>
<tr>
<td>&lt; 100</td>
<td>0% or 8 (whichever is greater)</td>
</tr>
<tr>
<td>100-500</td>
<td>20 Articles</td>
</tr>
<tr>
<td>500-1000</td>
<td>4% of 40 (whichever is less)</td>
</tr>
</tbody>
</table>

#### Sterilization ISO Standards

| ISO 11135 Ethylene Oxide Sterilization (Biological Indicator Qualification Study) | Follow USP sterility method |
| ISO 11137 Radiation Sterilization Procedure Method 1: 100 VDA 100 SDA Single Batch Sterility Test 100 Samples VDA (only) |  |
| VD<sub>Max</sub>: 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. VD<sub>Max</sub>
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.

8. Validation of garment sterilization.

9. 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.

1. Identification and speciation of the isolate is a significant contributing factor to the final decision of an action plan.
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 (ID’s)
   b. Media sterilization
   c. Training
   d. Sample procedure (level of difficulty)
   e. Controls

Conclusion:
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 (1).

References: