



Understanding the Role of Sterility Testing in Compounding

First appearing in the British Pharmacopoeia in 1932, the sterility test included the basic features of the test we use today: Two media, a prescribed dilution scheme, and a defined incubation time. Shortly thereafter, the USP also adopted a sterility test; the first version, which appeared in 1935, included one medium, a dilution scheme, and a retest provision to evaluate failures. Perhaps surprisingly, today's sterility test has changed little from its initial origins.

To conduct (or even consider performing) beyond use dating (BUD), it is key that pharmacy be familiar with the driving concepts behind sterility testing. This also holds true for facilities outsourcing their IV production and any accompanying BUD testing. With a clear understanding of the requirements for sterility testing, pharmacy can review sterility test data with confidence and be assured the test was performed in a compliant manner.

Test Design

USP Chapter <71> Sterility Tests is internationally harmonized and describes two separate types of tests—membrane filtration and direct inoculation. The membrane filtration method is preferred, but the direct inoculation method is also detailed in the chapter. Both methods use the same volume of compounded sterile preparation (CSP) for testing and two different microbial recovery media; one to

recover aerobic microorganisms, the other to recover anaerobic microorganisms that may be in the CSP. Specific incubation temperatures are delineated for the different media and a 14-day incubation period is employed for both approaches.

Membrane Filtration

Sterility testing through membrane filtration begins by filtering the required volume of CSP through two membrane filters (nominal pore size no greater than 0.45 μm). Next, a diluting or neutralizing fluid is passed through each filter to eliminate antimicrobial residue from the filter, usually over the course of three separate rinses. The first filter is then submerged in an aerobic microbial recovery broth (usually soybean casein digest medium—SCDM) and the second, identically treated membrane filter is placed into an anaerobic recovery broth (usually fluid thioglycollate medium—FTM). The separate samples are then incubated (SCDM at 20-25°C and FTM at 30-35°C) for 14 days. Any growth in the recovery media is evidence of viable cells in the CSP.

Direct Inoculation

The direct inoculation method of sterility testing is described in USP <71> as a secondary method should the membrane filtration method prove ineffective. For this approach the required volume of CSP (the same volume of CSP as for membrane filtration) is added directly to the recovery broth media (SCDM or FTM) and incubated, again at the specified temperatures for 14 days.

The required volumes of CSP are strictly defined and depend on several factors of the compounded preparation, including:

- CSP type
 - ▶ Standard
 - ▶ Antibiotics
 - ▶ Ophthalmic
 - ▶ Others (non-liquid, bulk powders, etc. See TABLE 1 of USP <71>)
- Volume of CSP filled per container
See TABLE 2 of USP <71>
- Number of units filled per batch
See TABLE 3 of USP <71>

Method Suitability Test

The challenge of conducting a sterility test is different for each specific CSP (defined by concentration of active(s), excipients, preservatives, as well as the method of preparation) and must be qualified by a method suitability test. The method suitability test serves as a positive control using different challenge microorganisms to demonstrate that the dilution/recovery scheme used is effective at neutralizing any residual antimicrobial properties of the CSP. The method suitability test also defines the methodology of a given sterility test. For example, a membrane filtration test will be defined (and have a method suitability test to qualify the test) in terms of:

- CSP (formulation, volume filled, number of units filled, method of preparation)

Alternate Sterility Tests

USP allows for alternate tests for any compendial method so long as that alternate method is demonstrated to be at least equivalent to the standard. Thus, the challenge for rapid or alternate sterility tests is to demonstrate equivalence to USP <71>. USP provides guidance on this in Chapter <1227> Validation of Microbial Recovery from Pharmacopoeial Articles, which first became official in 2006. However, the compounding pharmacy must be careful of the use of these methods, as the demonstration of equivalence to USP <71> is not an easy task, and beyond showing the method suitability required in any USP <71> compliant test, the alternate technology must also be demonstrated as acceptable. USP <1223> describes these requirements in terms of:

- ▶ **Specificity:** The test's ability to detect a range of microorganisms in the sample
- ▶ **Limit of Detection:** The lowest number of microorganisms that can be detected by the test
- ▶ **Ruggedness:** The performance of the test under a variety of normal test conditions
- ▶ **Robustness:** The capacity of the test to give consistent results under different method parameters

The alternate method is expected to perform at least as well as the traditional method in all of these comparative parameters.

- Filter used (filter material and nominal pore size)
- Diluent used (formulation of diluent, volume of diluent filtered per rinse and number of rinses)
- Recovery medium used (if SCDM or FTM is modified to enhance recovery)
- Microorganisms used—there are six separate microorganisms described in USP <71> for a compliant method suitability test. These all must be present, or a strong rationale must be provided for modification of these challenge organisms to conduct a compliant method suitability test.

Furthermore, there are some critical, non-negotiable aspects to a compliant USP <71> sterility test. These include strict compliance with compendial requirements for:

- Volume of CSP tested as defined by unit fill volume and batch size
- Demonstration of method suitability
- Recovery conditions (described in USP, defined by the method suitability test)
- Two media (SCDM and FTM)
- Specific incubation temperatures associated with each medium
- 14 days minimum incubation

It is important to note that the sterility test is not a perfect test. Its flaws include the fact that it can only recognize organisms able to grow under the conditions of the test. Another issue is that the sample size is so restricted that it provides only a gross estimate of the state of sterility of the product lot. As such, it is generally recognized that the test is inherently limited as a quality control test for finished product release. It is for this reason that contamination and process control of the compounding preparation is so important—these are the true safeguards of the sterility of the CSP.

The Sterility Test for CSP Release

Clearly the sterility test is not an ideal release test for sterile CSPs, and this is underscored by the frequently reinforced regulatory position that process control is critical to the sterility assurance of all CSPs. However, while acknowledging the limitations of the compendial sterility test, USP <797> continues to require this test under GCP (good compounding practice), especially for high-risk CSPs. The chapter clearly requires testing of:

- High Risk CSPs:
 - ▶ Sterilized in batches of more than 25 units; or
 - ▶ That is in multiple dose vials for administration to multiple patients; or
 - ▶ That is exposed longer than 12 hours at 2-8°C or longer than six hours at greater than 8°C before sterilization; or
- Any CSP utilizing BUD, regardless of risk level

The performance of the test in a compliant manner is therefore of critical importance for many CSPs prepared in normal compounding practice.

Reviewing Sterility Data

The sterility test report should provide sufficient information to allow you to confirm that the test was done in a compliant manner. As such, a clear reference to the relevant method suitability test for a specific CSP must be provided. The report also should include the volume of CSP tested (which is determined by the type of CSP, the number of units filled, and the volume of CSP per unit filled), the number of days the sample was incubated at each required temperature, and whether any growth was observed. The primary issue is that you receive sufficient documentation to confirm that each sterility test was performed in a compliant manner regardless of whether it was done for a high-risk CSP, BUD extension, or any other purpose.

A note of caution is necessary here—the sterility test is not an infallible indication of the sterility of that CSP batch. Far more important are ensuring the

Suggested Reading

- ▶ USP General Chapter <797> - Pharmaceutical Compounding—Sterile Preparations
- ▶ USP General Chapter <1163> - Quality Assurance in Pharmaceutical Compounding
- ▶ Conley C, Granko R. Outsourcing beyond-use dating validation. *Pharm Purch Prod.* 2013;10(6):16-17.
- ▶ Sutton SVW, Knapp JE, Porter D. Activities of the USP analytical microbiology committee of experts during the 2000–2005 revision cycle. *PDA J Pharm Sci Technol.* 2005;59(3):157-176.
- ▶ Sutton S, Tirumalai, R. Activities of the USP microbiology and sterility assurance expert committee during the 2005–2010 revision cycle. *Am Pharm Rev.* 2011;14(5):12-30.
- ▶ Sutton S. Validation of alternate microbiology methods for product testing – quantitative and qualitative assays. *Pharm Technol.* 2005;29(4):118-122.
- ▶ Sutton S. The Sterility Tests. In: Moldenhauer J, ed. *Rapid Sterility Testing.* Bethesda, MD: PDA/DHI Publishing; 2011:7-24.
- ▶ Sutton S. GMP and compounding pharmacies. *Am Pharm Rev.* 2013;16(3):48-59.
- ▶ USP. 1935. Sterility Test for Liquids. USP XI. p. 469.

effectiveness of the compounding process controls, environmental controls, and the training and expertise of the staff on aseptic manipulations. These processes can be assessed by reviewing the equipment in use and facility qualifications, such as smoke studies, media process simulations (ie, media fills), as well as other process controls described in USP <797>.

Conclusion

Sterility testing delineated in USP <71> is required for a significant number of CSPs routinely compounded under GCP as described by USP <797> and USP <1163>. This test is defined with detail in the USP and any modifications in the critical parameters of the test may imperil the compliant nature of the test results.

Alternatives to the sterility test are permitted in the compendia and Chapter <122> provides guidance for demonstrating the equivalence of alternate microbiological methods to the compendial method. Ultimately, it is prudent to understand the basics of sterility testing and to determine the compliance of the critical testing performed on your CSPs. As delineated in USP <1163>, it is clearly the responsibility of the compounding pharmacy to ensure the quality of the CSPs it produces (found under the section on Testing). With this in mind, gaining a better understanding of how sterility testing is performed is vital as an adjunct to good compounding practice and process control. ■



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