



LABORATORY CONSIDERATIONS OF *UNITED STATES*
PHARMACOPEIA CHAPTER <71>

Sterility Tests and its Application to Pharmaceutical Compounding



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ABSTRACT The purpose of this article is to describe *United States Pharmacopeia* Chapter <71> Sterility Tests from the perspective of Current Good Manufacturing Practices in order to aid compounding pharmacists in understanding the details and complexities that are required. Compounding pharmacists face a unique challenge in the industry today, with their compounding practice and the U.S. Food and Drug Administration trying to impose Current Good Manufacturing Practices guidelines. Naturally, this becomes a challenge to contract testing laboratories as well, as they are caught between the testing for non-Current Good Manufacturing Practices compounding standards and Current Good Manufacturing Practices manufacturing. It is important that the compounding pharmacist and their partner testing laboratory work closely together to ensure appropriate requirements are being met.

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A series of U.S. Food and Drug Administration (FDA) audits involving compounding pharmacies and contract testing laboratories over the last year has presented pharmaceutical compounding with some new challenges. During the audits, pharmacists and testing laboratories found themselves being inspected to the requirements of Current Good Manufacturing Practices (cGMPs). Not only are the FDA inspectors inspecting using a different and more strict set of guidelines, but the governing organizations (state boards of pharmacies) also are being more heavily scrutinized to ensure the safety of the public. The FDA's *Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing—Current Good Manufacturing Practice* references *United States Pharmacopeia (USP)* Chapter <71> Sterility tests as “the principle source used for sterility testing methods, including information on test procedures and media.”¹ It is important to understand the intent of the chapter and ensure all aspects of the chapter are followed including media preparation and quality, method suitability, and sampling requirements. From a cGMP perspective, it is also critical to ensure that adequate investigations and documentation are occurring. Understanding the timeline for sterility procedures and how it might affect batch release also is helpful in the compounding pharmaceutical business.

The intent of *USP* Chapter <71> is often misinterpreted to indicate that a passing result ensures that a batch of product is sterile although the chapter clearly states, “These Pharmacopeial procedures are not by themselves designed to ensure that a batch of product is sterile or has been sterilized.”² The true intent of the chapter is to demonstrate “that the material tested meets the requirements of the test.”³ This fact is also clearly stated within *USP* Chapter <71>, “...a satisfactory result only indicates that no contaminating microorganism has been found in the sample examined under the conditions of the test.”²

MEDIA PREPARATION AND QUALITY

USP Chapter <71> provides the formula for Fluid Thioglycollate Medium as well as Soybean-Casein Digest Medium. The guidance chapter also allows for the use of equivalent commercial media as long as the media meets the requirements of the growth promotion test for the organisms specified in the chapter. “The quality of work in a microbiological laboratory depends on the quality of the culture media” and “the quality control of the media is a critical concern.”⁴ When performing sterility testing, laboratories must perform quality-control tests to ensure growth media meets the requirements described in the *USP*. For *USP* Chapter <71>, this includes confirming sterility and growth promotion testing for each lot of

AT - A - GLANCE

Requirements for meeting cGMP and USP <71> Sterility Tests

- **MEDIA PREPARATION AND QUALITY**
 - > Growth promotion testing and sterility
- **METHOD SUITABILITY**
 - > Based on product formulation
 - > Utilize and recover the 6 organisms describes in *USP* <71>
- **SAMPLING**
 - > Refer to Tables 2 and 3 from *USP* <71>
- **INVESTIGATIONS AND DOCUMENTATION**
 - > Both must be thorough, complete and traceable

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media. In order to confirm sterility, portions of the media are incubated for 14 days during which time no growth should occur. In order to satisfy the requirement of growth promotion testing, six challenge organisms must be inoculated into the appropriate medium in a quantity of <100 colony forming units. The media is then incubated within the specified temperature range, and growth must occur within the specified period of time. Documentation of these activities should adhere to the documentation requirements of cGMP.

METHOD SUITABILITY

One of the most important aspects of USP Chapter <71> is method suitability. Many preparations that must meet the requirements of the sterility test contain antimicrobial activity such as antibiotics or compounded sterile pharmaceuticals containing preservatives. An appropriate method for these preparations will ensure that antimicrobial activity has been sufficiently removed under the conditions of the test. It is important to repeat method suitability any time there is a change in the preparation formula-

A failing sterility test result is something no one likes, yet it is critical that “when microbial growth is observed, the lot should be considered nonsterile and an investigation conducted.” Compounders must perform a thorough investigation in order to obtain information that might aid in the decision of batch release once all investigatory work is complete.

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tion, such as an increase in the concentration of the active ingredient or the use of a different class of antimicrobial. For this reason, pharmacists must communicate any change in a preparation formulation to the testing laboratory so that changes in the method can be evaluated. Method suitability is also repeated any time a change in the test method is required. For instance, one may decide that membrane filtration is more appropriate due to the volume of the sample that is being tested. When large sample volumes are being tested, the method of direct inoculation can require very large volumes of media and incubator space. Each formulation must undergo method suitability testing, which involves testing the formulation by the method being utilized for the sterility test and inoculating the sterility test preparation with the six challenge organism as described in USP Chapter <71>. There are tens of thousands of unique formulations being prepared by compounding pharmacists, each one requiring method suitability testing utilizing the exact conditions in which the sterility test will be performed. In order to ensure a validated method is used for the sterility testing of a product, it is helpful to perform method suitability on a formulation before undergoing the actual sterility test for a batch.

SAMPLING

The sampling of articles is the most discussed and documented limitation of the sterility test. For this reason, sampling the batch appropriately is a critical part in ensuring that everything possible is done to ensure that the preparation is safe to dispense.⁵ Where sampling is concerned, “it is important that the samples represent the entire batch and processing conditions.”¹ This is often inter-

preted as taking samples from the beginning, middle, and end of the process. Articles to be tested from the finished batch contain the final product formulation in the products final container/closure. Intermediate steps can and should be tested for sterility but *USP* Chapter <71> was not written and is not intended for this purpose. *USP* Chapter <71> contains two tables that describe sampling. Table 3 specifies the number of articles to be tested based on the batch size and Table 2 specifies the quantity to be tested from each of the articles when quantities are sufficient to ensure equal portions are added to each media. "If each article does not contain sufficient quantities for each medium, use twice the number of articles indicated in Table 3."² A sufficient quantity is described as greater than or equal to 2 mL according to Table 2. This means that an article containing less than 2 mL requires twice the number that is described in Table 3 for sterility testing. It is important to understand that a batch contamination can occur in an individual article or throughout the batch, which can be defined as the frequency of contaminated units in a batch and can significantly impact the results of the sterility test depending on how sampling is performed.

It should be recognized that the referee sterility test might not detect microbial contamination if present in only a small percentage of the finished articles in the lot because the specified number of units to be taken imposes a significant statistical limitation on the utility of the test results...This inherent limitation, however, has to be accepted, because current knowledge offers no nondestructive alternatives for ascertaining the microbiological quality of every finished article in the lot, and it is not a feasible option to increase the number of specimens significantly.¹

INVESTIGATIONS

A failing sterility test result is something no one likes, yet it is critical that "when microbial growth is observed, the lot should be considered nonsterile and an investigation conducted."¹ Compounders must perform a thorough investigation in order to obtain information that might aid in the decision of batch release once all investigatory work is complete. Essential during the investigation is a review of the batch preparation records. It is also important to understand where the

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potentials for batch contamination can occur; define and validate your processes to ensure that the potential for a contamination in your product is low. "Appropriate written procedures, designed to prevent microbiological contamination of drug products purporting to be sterile, shall be established and followed. Such procedures shall include validation of all aseptic and sterilization processes."⁶ An understanding of your validated process, written procedures for executing your processes, and understanding the limitations of each part of the process, especially product testing, can go a long way in accomplishing an investigation that meets the requirements of cGMPs such as¹:

- Identification of the organism in the test
- Record of laboratory tests and deviations
- Monitoring of production area environment
- Monitoring personnel
- Product presterilization bioburden
- Production record review
- Manufacturing history

TABLE 1. Minimum Quantity to be Used for Each Medium.

QUANTITY PER CONTAINER	MINIMUM QUANTITY TO BE USED (UNLESS OTHERWISE JUSTIFIED AND AUTHORIZED)
LIQUIDS	
Less than 1 mL	The whole contents of each container
1-40 mL	Half the contents of each container, but not less than 1 mL
Greater than 40 mL, and not greater than 100 mL	20 mL
Greater than 100 mL	110% of the contents of the container, but less than 20 mL
Antibiotic Liquids	1 mL
Insoluble preparations, creams, and ointments to be suspended of emulsified	Use the contents of each container to provide not less than 200 mg
SOLIDS	
Less than 50 mg	The whole contents of each container
50 mg or more, but less than 200 mg	Half the contents of each container, but not less than 50 mg
300 mg-5g	150 mg
Greater than 5 g	500 mg
Catgut and other surgical sutures for veterinary use	3 sections of a strand (each 30-cm long)
*Surgical dressing/cotton/gauze (in packages)	100 mg per package
Sutures and other individually packaged single-us material	The whole device
Other medical devices	The whole device, cut into pieces or disassembled*

* If the batch size is unknown, use the maximum number of items prescribed.

Contract testing labs must also perform a thorough investigation when positive sterility test results are obtained. Bacterial identification of the sample contaminate(s) and comparison to the bacterial identification of environmental isolate(s) from the testing facility and personnel is often a key piece of evidence. It can provide information that can aid in the decision of whether or not a retest should be performed. "Care should be taken in the performance of the sterility test to preclude any activity that allows for possible sample contamination" such as environmental monitoring and training of personnel in aseptic processes.¹ Although many precau-

TABLE 3. Minimum Number of Articles to be Tested in Relation to the Number of Articles in the Batch

NUMBER OF ITEMS IN THE BATCH*	MINIMUM NUMBER OF ITEMS TO BE TESTED FOR EACH MEDIUM (UNLESS OTHERWISE JUSTIFIED AND AUTHORIZED)**
PARENTERAL PREPARATIONS	
Not more than 100 containers	10% or 4 containers, whichever is the greater
More than 100 but not more than 500 containers	10 containers
More than 500 containers	2% or 20 containers, whichever is less
*For large-volume parenterals	2% or 10 containers, whichever is less
ANTIBIOTIC SOLIDS	
Pharmacy bulk packages (<5 g)	20 containers
Pharmacy bulk packages (≥5 g)	6 containers
Bulks and blends	See Bulk solid products
OPHTHALMIC AND OTHER NONINJECTABLE PREPARATIONS	
Not more than 200 containers	5% or 2 containers, whichever is the greater
More than 200 containers	10 containers
If the product is presented in the form of single-dose containers, apply the scheme shown above for preparations for parenteral use.	
Catgut and other surgical sutures for veterinary use	2% or 5 packages, whichever is the greater, up to a maximum total of 20 packages
*Not more than 100 articles	10% or 4 articles, whichever is greater
More than 100, but not more than 500 articles	10 articles
More than 500 articles	2% or 20 articles, whichever is less*
BULK SOLID PRODUCTS	
Up to 4 containers	Each container
More than 4 containers, but not more than 50 containers	20% or 4 containers, whichever is greater
More than 50 containers	2% or 10 containers, whichever is greater

* If the batch size is unknown, use the maximum number of items prescribed.

** If the contents of one container are enough to inoculate the two media, this column gives the number of containers needed for both the media together.

Audits should be performed as well as a review of all processes and procedures to ensure they are meeting the appropriate standards, “...for example the documentation and maintenance of laboratory records.” It is not enough to assume this is being done and leave the responsibility solely on the laboratory. It is best to act proactively and ensure documentation is being performed to meet all requirements.

tions are taken to avoid false sterility test positives, it is important to recognize the technical limitations of sterility testing.⁷

DOCUMENTATION

Documentation is extremely important for cGMP compliance. Documentation for cGMP must contain details of information such as equipment used, lot numbers, expiration dates, etc. Documentation that is associated with all equipment and materials must also be traceable, such as calibration and cleaning information, manufacturer and receipt information, as well as any testing that must be performed on starting materials as part of the documented procedure. It is important to follow the procedures exactly as they are written and have the documentation to prove it. When procedures are not followed or there are errors that occur, deviations and corrective actions must also be documented and traceable. Compounding pharmacies should work closely with their contract testing laboratories to ensure their partner laboratory is meeting their testing requirements and expectations. Audits should be performed as well as a review of all processes and procedures to ensure they are meeting the appropriate standards, “...for example the documentation and maintenance of laboratory records.”⁴ It is not enough to assume this is being done and leave the responsibility solely on the laboratory. It is best to act proactively and ensure documentation is being performed to meet all requirements.

TIMING

The sterility test requires a minimum 14-day incubation period and oftentimes requires additional sub-culturing and incubation when the properties of the product being testing cause the media to become turbid for reasons other than as a result of microbial growth. Naturally, this can make reading the sterility test result difficult, and an additional investigation, such as microscopic examination, may be required in order to ensure the accuracy of the ste-



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rility test. “The currently required 14-day incubation period imposes a significant burden on the manufacturer, who must quarantine product until successful completion of the test.”⁸ In addition to this, there are two critical aspects that can significantly increase the timing required to complete a sterility test, which are 1) method suitability and 2) investigations. Method suitability may be performed simultaneously with the test for sterility, but obtaining method suitability for the formulation prior to testing the product for sterility can aid in the prevention of this delay. If the method suitability test fails, you must perform method suitability again and repeat the sterility test by the new method. Naturally, this causes delays in obtaining sterility test results and can have an impact on the release of a batch. Also critical and timely, are investigations. If, for instance, your sterility test result was determined to be a laboratory error and you must repeat your sterility test to confirm your original invalidated test result, you could potentially be at least 6 to 8 weeks beyond the date when the original sterility test was initiated before you receive the final test results. A closer look at how an investigation might play out over time can help in understanding the issue:

1. Turbidity is observed in the sterility test on the 14th day of incubation.
2. Sample contaminate is isolated by plating onto TSA and incubating; this can be 1 to 6 days.
3. Sample contaminate is compared morphologically to environmental and personnel isolates.
4. Bacterial identification of the species are obtained; this can take 3 to 10 days.
5. Retest is performed of the full 14-day sterility test.

Timing of the sterility test is no doubt a burden, but understanding that delays can occur and the timeline of the delays may help to ensure processes are in place to minimize this inevitable burden.



CONCLUSION

With the changes currently occurring in the pharmaceutical compounding and testing industry, we are all facing unique challenges in learning to balance the traditional needs for compounded pharmaceuticals, preparing these products on a larger scale to address drug shortages, and how to best protect the public. One of the most challenging aspects is to understand the intent of USP Chapter <71> and being able to apply this information to the data and results obtained from the sterility test. cGMP guidelines require additional detail to all aspects of the sterility test as well as ensuring compliance of the procedures and documentation. Testing now requires compounders and their partner testing laboratories to work collaboratively.

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