

# Quality-Control Analytical Methods: *USP* Chapter <797> Compounded Sterile Preparations Sterility Requirements and Their Relationship to Beyond-Use Dating

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## Introduction

The *US Pharmacopeia-National Formulary (USP-NF)* has been setting standards since 1820; it has been setting official enforceable standards since 1906. Originally, all of the standards applied to pharmacy compounding, but in the mid-1900s emphasis moved toward standards for the pharmaceutical industry. With the recent increase in pharmacy compounding, the *USP* has introduced specific chapters, monographs and standards in the official compendia for pharmacists to support quality compounding.

One area of the new *USP* Chapter <797> “Pharmaceutical Compounding—Sterile Preparations” is causing confusion and concern for pharmacists and technicians both in determining beyond-use dating (BUD) and in meeting the sterility requirement. The creation of a three-risk-level classification system and strict assignment of BUD for compounded sterile preparations (CSPs) are based on the potential for chemical and physical stability and microbial contamination. Monitoring sterility of processes and CSPs is in an integral part of any quality plan.

The BUDs discussed in *USP* compounding Chapters <795> and <797> are related as follows. If sterility testing is not performed, the BUDs provided in Table 1 cannot be exceeded. If sterility testing is performed, the BUD can be determined from the chemical and physical stability of the final CSP according to scientific studies,<sup>1</sup> published references<sup>2</sup> or personal correspondence with manufacturers, or the same BUDs as for nonsterile preparations apply as listed in

**Table 1. Beyond-Use Dates for Compounded Sterile Preparations Without Sterility Testing.**

Risk level	Room temperature	Refrigeration	Freezer ( $\leq -20^{\circ}\text{C}$ )
Low	48 hours	14 days	45 days
Medium	30 hours	7 days	45 days
High	24 hours	3 days	45 days

**Table 2. Beyond-Use Dates for Compounded Sterile Preparations With Sterility Testing and for Nonsterile Preparations.**

Nonaqueous liquids and solids	
Manufactured drug product is source of drug	25% of time remaining until the preparations expiration date, or 6 months, whichever is earlier
<i>USP-NF</i> substance is source of drug	Not later than 6 months
Water-containing formulations	Not later than 14 days when stored between $2^{\circ}\text{C}$ and $8^{\circ}\text{C}$
All other formulations	Not later than the intended duration of therapy or 30 days, whichever is earlier

*Note: Table 2 represents BUDs that can be used in the absence of stability information that is applicable to a specific drug and preparation, when stored in tight, light-resistant containers at controlled-room temperature, unless otherwise indicated.*

*USP-NF = United States Pharmacopeia-National Formulary*

*USP* Chapter <795> and as detailed in Table 2. It is the professional responsibility of the pharmacist to make the final decision as to the BUD requirements for their various compounded preparations.

The two factors that are critical in establishing BUD (formerly known as expiration dating) for CSPs are as follows:

1. *The chemical stability of the chemical entity in solution.* There are numerous textbook references as well as journal articles addressing the chemical stability and compatibility of medications in solution.
2. *The sterility of the CSP.* By definition, sterility is the absence of viable microorganisms in the CSP. Unlike pyrogenicity, sterility is an absolute concept. A CSP is either sterile or it is not. Although *sterility* is an absolute term, the assurance that any

given item is sterile is a probability function, commonly expressed as a negative power to the base ten.

## Principles of Sterility

It is important to understand the principles of sterility by understanding the difference between sterility assurance levels (SAL) and rates of contamination. As mentioned previously, the definition of sterility is the absence of viable microorganisms. Rates of contamination involve the number of compounded preparations that are nonsterile within a batch after preparation. *Sterility assurance level* is defined as the probability of an item being nonsterile after it has been exposed to a validated sterilization process (validated filtration, steam, ionizing radiation or ethylene oxide). Terminally sterilized preparations typically

have an SAL of  $10^{-6}$  (the probability of one in a million compounded and sterilized items being nonsterile).

Preparations compounded via aseptic procedure (no final filtration or terminal sterilization) can *only* claim an SAL of no more than  $10^{-3}$  (the probability of one in a thousand items being nonsterile), assuming that a robust compounding procedure (ongoing properly validated process) has been conducted. The importance of validating aseptic compounding processes cannot be overemphasized; validation is critical to support any assigned BUD and can be done through media fills. The best that can be hoped for with CSPs that are prepared without being terminally sterilized is a contamination no less than 1 contaminated unit per 1000 compounded units. This is why all other supportive functions and activities (eg, employee training, facility design, cleaning, gowning and gloving, validated processes) are so vitally important to the sterility of the final CSP. Statistically speaking, SAL can be determined and verified through process and employee aseptic process validation using tryptic soy broth. If a pharmacy operation can prepare 3,000 media fill units or more without a positive media fill (*no growth*), it can be claimed with 95% confidence that the rate of contamination of the processes and the people who compound is not greater than 0.1% or 1 CSP per 1,000 compounded units.<sup>3</sup>

## The Role of Process Validation in Establishing BUD and CSP Sterility

The sterility and quality of compounded sterile preparations are dependent upon a number of factors, including:

- The types of components (sterile versus nonsterile)
- The methods of compounding (open versus closed systems)
- Controlled compounding environment
- The use of engineering controls (eg, laminar airflow workbenches, isolators, cleanrooms)
- The controls (eg, calculations, double-check system, batch record documentation) during compounding
- Final preparation testing

Due to the complexity of compounded sterile preparations, performing *only* routine final preparation testing is often not sufficient to assure sterility and quality for several reasons.<sup>4</sup>

These issues include:

- Insufficient sensitivity to verify sterility and quality of the compounded preparation (eg, antibiotics or cytotoxic agents)
- Destructive testing to show that compounding produced a sterile and correct preparation (absolute sterility cannot be practically demonstrated without testing every unit within a batch)<sup>5</sup>
- Poor compounding techniques or errors that can yield a false-positive result rather than detect a nonsterile preparation
- Routine final preparation tests that do not reveal all variations in quality and sterility [eg, visually inspecting total parenteral nutrition (TPN) or other compounded preparations]

Process validation involves establishing documented evidence that a specific compounding procedure will consistently result in a final compounded preparation that meets its predetermined specifications of sterility with a high degree of confidence.<sup>6</sup> Sterility and final preparation testing has scientific and practical limitations that are well known. Some of these are:<sup>6</sup>

1. The statistical limitations of the sample size used for testing in any testing program also apply to sterility testing;
2. Final preparation testing may not be a definitive and useful method of assuring quality;
3. Sterility tests will only detect viable microorganisms present at the time of the test;
4. Viable organisms present at the time of the test can only be detected if they are capable of growth in the specified culture media;
5. Sterility and pyrogen tests may be subject to potential interference from drug concentrations;
6. Adventitious microbial contamination introduced at the time of testing may result in false-positive readings; and
7. Sterility tests are always destructive of the samples tested and do not offer the opportunity to re-examine the same samples in the event of either positive or negative findings.

It is important to note that according to *USP Chapter <71>*, "...these procedures [Sterility Tests] are not by themselves designed to ensure that a batch of product is sterile or has been sterilized. This is accomplished primarily by validation of the sterilization process (the use of biological indicators for steam sterilization cycles) or of the aseptic processing procedures." Additionally, the sterility tests are limited in their ability to

### Continuing Education

#### GOALS AND OBJECTIVES

"Quality-Control Analytical Methods: *USP Chapter <797> Compounded Sterile Preparations Sterility Requirements and Their Relationship to Beyond-Use Dating*"

*Goal:* The goal of this presentation is to provide compounding pharmacists with supportive information on the relationship of sterility testing and beyond-use dating when compounding sterile preparations.

*Objectives:* After reading and studying the article, the reader will be able to:

1. Discuss the principles of sterility and sterility testing.
2. Describe the factors affecting sterility and quality of compounded sterile preparations.
3. Discuss the scientific and practical limitations of sterility and final preparation testing.
4. Describe the direct inoculation and membrane filtration sterility testing methods.
5. Determine the number of units of compounded sterile preparations that should be tested in a batch.

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detect whether a batch contains contaminated units, with a threshold sensitivity of 15%. This means that 15% of a batch (no less than 2 bags per batch of 10 bags) would have to be contaminated and tested to detect the presence of contamination. Notwithstanding this limitation, sterility testing and ensuring the sterility of the CSP are critical to the patient's health and safety and a requirement of *USP* <797>.

Since patient injury and death following administration of CSPs have been associated with microbial contamination, the assignment of risk levels and the operating principles outlined in *USP* Chapter <797> serve to prevent CSP microbial contamination. Unless sterility testing is performed for the CSP, the BUD of the preparation *cannot* exceed the published limits found in *USP* <797> (Table 1). If sterility testing is performed according to *USP* <71> "Sterility Tests," the CSP can be assigned a BUD based on the chemical stability of the drug as presented in *USP* Chapter <795>. With these limitations and challenges, how does one meet the BUD limitations without testing and/or sterility requirements set forth in the chapter or according to state board pharmacy practice acts?

### **USP <71> "Sterility Tests"**

As noted earlier, final preparation sterility testing (microbial testing) as a quality release standard has many limitations. Notwithstanding these limitations, *USP* standards and state board of pharmacy regulations require some form of sterility (microbial) testing. Despite the limitations, microbial testing has two roles in a sterile preparation compounding program: (1) testing of preparations suspected of contamination, and (2) testing of batch-compounded preparations that are quarantined before use.

### **Methods of Testing**

Whether sterility tests are outsourced or done in-house, the sterility requirements outlined in *USP* Chapter <71> need to be followed. Any contract laboratory used by compounding pharmacists should ensure they comply with this chapter. A brief summary of the chapter follows.

To meet the requirements of *USP* Chapter <71>, two different growth media have to be used. They are (1) fluid thioglycollate medium (FTM), and (2) soybean casein digest medium (SCDM) (formerly trypticase soybean broth). FTM is primarily intended to culture anaerobic and aerobic bacteria. SCDM is primarily intended to culture fungi and aerobic bacteria. The test microorganisms used to validate the growth promotion ability of the sterility test media include *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Clostridium sporogenes* and *Candida albicans*; specific ATCC (American Type Culture Collection) strains are listed in *USP* <71>.

There are two official methods of microbial testing: (1) direct inoculation (direct transfer) of a sample to sterile culture media, and (2) membrane filtration. These methods are described later but should be read in the *USP 27-NF 22* Chapter <71>.

### **Direct Inoculation**

With direct inoculation, a small sample from a large volume solution is taken, then added to a growth medium and incubated. A sample aliquot of a compounded parenteral preparation is aseptically transferred into two sterile culture media. One medium, FTM, is incubated at 32°C for 14 days. The other medium, SCDM, is incubated at 22°C for 14 days. Positive (intentionally contaminated samples with known organisms) and negative (no contamination) controls are incubated with the test samples. Any sample that becomes turbid during the incubation period is a positive test. This technique can detect only *grossly* contaminated preparations. It might be used to test a quarantined batch compounded significantly in advance of use or a suspected preparation several days after compounding. Sterile culture media testing is of limited use for evaluating individual compounded sterile preparations within a few hours of compounding.

Turbidity in the growth medium indicates contamination. There are drawbacks to direct inoculation. If the product was a milky or turbid product, it could cause the medium to appear turbid, making the detection of microorganism growth difficult. Also, due to inherently low levels of contamination in the solutions, direct inoculation is not necessarily sensitive enough to reflect accurately the condition of the batch. For instance, a 1-mL sample from a 1000-mL container would only detect a contamination level of over 1 organism per mL. If the container only had 10 organisms, then the direct inoculation method would have a minimal chance of detecting the contamination.

### **Membrane Filtration**

Membrane filtration is preferred over direct inoculation for several reasons. The method does not depend on product type, container volume or concentration of microorganisms to provide a statistically valid sample. With membrane filtration the entire volume can be filtered, which captures all of the microorganisms present, removes product components that could cause the growth medium to appear turbid and can decrease the inhibition of microorganism growth due to antimicrobial agents in the preparation because they are filtered away during this method. The nature of the membrane filter method reduces operator handling, which decreases the likelihood of accidental contamination. This is ideal for most compounded preparations [small-volume parenterals (SVP), large volume parenterals (LVP), emulsions, suspensions and antibiotics].

When using this method, the entire preparation is filtered through an appropriate sterile 0.45- $\mu$ m filter. The membrane is washed by filtering a sterile fluid to remove compounds having a bacteriostatic effect. The membrane is aseptically divided and placed into FTM and SCDM and incubated at 32°C and 22°C, respectively. Both positive and negative controls are used. Turbidity during the 14-day incubation is a positive test.

All sterility testing for microbial contamination must be performed in an International Organization for Standardization (ISO) Class 5 (formerly known as Class 100) environment, with the use of proper aseptic technique to prevent

unintended environmental and operator contamination. The drug product lot from a US Food and Drug Administration (FDA)-registered manufacturer would fail the *USP* test requirement if any microbial growth is found and the test is not invalidated. The *USP* states that a manufacturer should not perform sterility retest without evidence that a positive sterility test can be attributed to contamination introduced by the laboratory operator's technique.<sup>7</sup>

### What Has to Be Tested?

If the BUD of the low-, medium- or high-risk level CSP does not exceed the published dating in *USP* Chapter <797>, sterility testing is not required except for high-risk-level batches of more than 25 units. Because of the varied practice settings and compounding procedures, *USP* Chapter <797> was written in the broadest terms using general descriptive states. As such, no single "iron-clad" determination of risk exists. Risk level classification in Chapter <797> is, in general, not prescriptive, with one exception: *CSPs prepared from bulk, nonsterile components are a high-risk level procedure as described in Chapter <797>*. Assigning the appropriate risk level to a CSP requires the professional judgment of the pharmacist.

The chemical stability of most CSPs will be greater than the *USP* published dating and, as such, undergo sterility

testing unless they meet the following conditions:

1. Compounded preparations: Such preparations will be used prior to *USP* BUD.
2. Single-unit, single-batch: There are examples that preclude routine sterility testing. They include:
  - a. TPN solutions prepared in the hospital or on a daily basis are not batched and typically do not exceed the 30-hour room temperature stability for a medium-risk-level CSP. As such, no sterility testing is required.
  - b. Single syringes, cassettes, SVP or LVP bags of chemotherapy, pain management preparation: Although these dosage units will be infused for periods greater than BUD for that risk-level compounding, they are not prepared in quantities that can be sterility tested. It is strongly recommended that other methods of sterility assurance be used for CSPs that are prepared from bulk nonsterile powders and then filtered. Suggested other methods are:
    - i. Use filters that are approved for human-use applications in sterilizing pharmaceutical fluids and certified by the manufacturer to retain at least 10<sup>7</sup> microorganisms of a strain *Brevundimonas diminuta*. Not all 0.2- $\mu$ m filters meet this requirement.
    - ii. Test the final filter via a filter integrity or "bubble-point" test to ensure that the filter sterilized the fluid according to manufacturers' specifications and did not fail.
3. Cytotoxic or other hazardous compounds may not be candidates for sterility testing (because of the nature and volume of the compounded preparation) *but* it is important to understand that there are reports that microorganisms like *E. coli*, *S. aureus* and *Pseudomonas* will grow in cytotoxic medication.
 

Multiple units, single-batch CSPs, TPN, antibiotics or other CSPs prepared in bulk or in quantities necessary to fulfill an order for a one-week supply would require sterility testing if the BUD exceeds 7 days refrigeration. An important term that needs to be defined here is *batch*. Is a batch the number of bags in one patient's order, all the bags compounded by one employee or all the bags compounded in one day? This definition must be determined by the pharmacist because, as stated previously, sterility testing is a destructive test and the CSP cannot be recovered.

### How Many Units Have to Be Tested?

Once the definition of batch size has been determined, *USP* <71> "Sterility Test" details the minimum number of items recommended to be tested to meet the requirements of *USP* <71> "Sterility Test" (Table 3 and Table 4). One of the only commercially available closed-system sterility testing systems is made by Millipore Corporation, Billerica, Massachusetts. Using the preferred method of sterility testing (membrane filtration versus direct inoculation), all units to be tested would be simultaneously run through two 47-mm, 0.45- $\mu$ m filter canisters.<sup>8</sup> This procedure would be followed by filling

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**Table 3. Recommended Number of Items to Be Tested for Sterility from Batches of Various Types of Preparations.**

Number of items in the batch	Minimum number of items recommended to be tested
<i>Injectable Preparations</i>	
Not more than 100 containers/articles	10% or 4, whichever is greater
More than 100 but less than 500 containers	10 containers
More than 500 containers	2% or 20 containers, whichever is less
<i>Large-Volume Parenterals</i>	
<i>Ophthalmic and Other Noninjectable Preparations</i>	
Not more than 200 containers	5% or 2 containers, whichever is greater
More than 200 containers	10 containers
<i>Bulk Solids</i>	
Less than 4 containers	Each container
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

**Table 4. Summary of USP Chapter <71> Parameters and Criteria.**

Parameter	Criteria
Facility	Cleanroom or isolators: Addresses different products (devices, topical, etc.)
Media	FTM and SCDM: Special procedure for media with penicillin or cephalosporins (ensures against inhibition of culture) and specifies validation of quantity of $\beta$ -lactamase to add
Incubation time	14 days: FTM 32.5° ± 2.5; SCDM 22.5° ± 2.5
Assay	No allowance for retest
Sample requirements	Bulk and final testing
Negative controls	Incubate for 14 days: Requires validation to ensure media is not fungistatic or bacteriostatic (inhibition)
Growth promotion	Specifies number and type of organisms to grow: Must grow within 5 days
Inoculum	Specifies volumes; specifies number of articles (ratios) to be tested; three inoculation methods; recommends membrane filtration method whenever possible
FTM = fluid thioglycollate medium	
SCDM = soybean casein digest medium	

one of the filter canisters with 100 mL of SCDM and the other with 100 mL of FTM. They would be incubated for 14 days (SCDM at 25°C and the FTM at 30°C–35°C) and visually inspected for any growth. In the event of a positive test, turbidity, cloudiness or floating particles, the positive canister should be sent to a microbiological laboratory to identify the species of the microorganisms. A positive finding is a serious matter, and an extensive investigation must be undertaken to identify the point of failure, if possible. According to USP test requirements, any drug product batch that fails a sterility test should be considered contaminated. All units should be recalled, if possible, if the test

is not invalidated. The USP 27-NF 22 states that a firm should not perform a sterility retest without evidence that the positive sterility test can be attributed to contamination introduced by the laboratory.<sup>9</sup> It is also critical to initiate a CSP recall and patient impact investigation to ensure that there was no patient harm as a result of the contaminated batch of CSP.

### Putting the Patient First

The microbiological sterility and chemical stability of the CSPs are crucial aspects of a pharmacist's and technician's job responsibility. There are several excellent resources and references available to pharmacists for

determining the chemical stability of CSPs. The microbiological sterility can be achieved through proper employee training, compounding facility design, construction, operation, control and maintenance, good aseptic techniques and habits, process validation and verification of aseptic compounding procedures. Sterility testing of CSPs is only required by USP Chapter <797> when the published BUDs are exceeded. Controlling the variables that can affect CSP sterility and stability, can assure the patient of receiving a properly compounded sterile and nonsterile preparation.

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