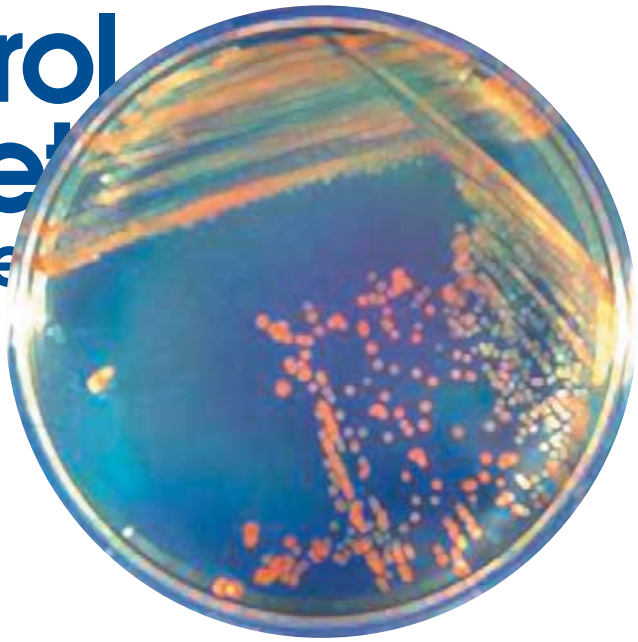
Quality-Control Analytical Me

Microbial-Testing Aspe of *USP* Chapter <797> for Compounded Sterile Preparations



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The standards set forth by the *United States Pharmacopeia (USP)* Chapter <797>[1] have now been in effect since January 1 of 2004. As the first practice standards of sterile pharmacy compounding in US history, they have "attracted both respect and criticism" because they have also been cited as a practice expectation by the Joint Commission on Accreditation of Healthcare Organizations.[2] *United States Pharmacopeia* Chapter <797> expands the scope of facilities governed by the regulations and defines the practices covered, emphasizing the importance of environmental quality and control, verification of accuracy and sterility, training and evaluation, quality control after preparations leave the pharmacy, patient monitoring and adverse events reporting.[3] The purpose of this article is to help the reader understand the criteria set forth by *USP* Chapter <797> regarding finished-product testing, including criteria for the microbial-testing aspects of sterility testing (*USP* Chapter <71>)[4] and endotoxin (pyrogen) testing (*USP* Chapter <85>).[5]

Sterility Testing

A compounded sterile preparation is an extemporaneously prepared medication of appropriate potency and purity, prepared using aseptic technique free of pyrogens and microorganisms. There are two methods of sterility testing, according to the *USP* Chapter <71>:[4] one is the direct-transfer method, and the other is the membrane-filtration method. Direct transfer is the most common or preferred. Membrane filtration requires a large sample size, more media than the direct-transfer methods and additional equipment. Therefore, direct transfer is more user friendly for compounding pharmacists.

Two forms of media can be used for direct-transfer testing: broth and agar. Broth is useful for clean and nonprecipitating solutions; agar is more useful for suspensions. The three types of media used to perform sterility testing are tryptic soy broth, fluid thioglycolate and sabouraud dextrose agar and/or potato dextrose agar (see Table 1 for the uses of each medium, temperature for testing and incubation period). While tryptic soy broth is a good general medium, fluid thioglycolate is more specific for anaerobic and sabouraud dextrose agar is more specific for fungi. *Note: Special care should be taken to test for fungi. Spores can lie dormant in vials and will not show up unless testing is performed in a special medium at a certain temperature.*

Table 1. Overview of Sterility Testing.

Media	Temperature (°C)	Purpose	Incubation Period
Tryptic soy broth (soybean casein)	22.5 ± 2.5 32.5 ± 2.5	Fungi, aerobic bacteria Anaerobic bacteria	14 days 14 days
Fluid thioglycolae	32.5 ± 2.5	Anaerobic bacteria	14 days
Sabouraud dextrose agar/potato dextrose agar	22.5 ± 2.5	Broad range of fungi	28 days

Membrane filtration can be used for special cases, such as antibiotics and heavy suspensions. The membrane-filtration and broth media show turbidity for positive samples, while agar forms visible colonies that can be counted to determine the level of contamination. Microscopic examination of the broth and agar is recommended to confirm the presence of organisms.

It is imperative to perform both a positive and a negative control during sterility testing or to ensure that an outside testing lab is using both controls. It is also important to validate the autoclave in use, which can be accomplished using bioindicators (spores that, when they fail to grow, demonstrate that the autoclave works), temperature probe and tape. To validate the autoclave, it must reach and maintain 121°C for at least 15 minutes to kill microorganisms.

If making your own media, you should perform media-growth-promotion verification. To perform media-growth-promotion verification requires spiking the media with the following bacteria to demonstrate growth: *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger* and *Clostridium sporogenes*. This will serve as a positive control. It is also necessary to incubate media to demonstrate no growth, which will serve as a negative control. This will avoid a false-positive result. To be in compliance with *USP* Chapter <71>,[4] media should be incubated for 14 days to ensure that there has been no growth. Additionally, the majority of organ-isms will grow within 48 hours.

There are several concerns regarding sterility testing. For example, it is possible to get both false-positive and false-negative results. A false positive could result when personnel contaminate media, or media are already contaminated. This is why it is very important to confirm that media are negative for growth before beginning testing.

A false negative can result in the media's being unable to grow an organism. This can result from a testing error from (1) using an incorrect sample, (2) not incubating media for sufficient time and/or (3) incubating media at too low a temperature. This is why it is important to perform media-growth-promotion verification to avoid false negatives and to incubate negatives to avoid false positives. It might be useful to evaluate the cost of sterility testing done inhouse vs. that done by an independent laboratory. If you choose to perform inhouse testing, it would be prudent to validate using an independent laboratory.

Endotoxin (Pyrogen) Testing

Endotoxins are most commonly associated with proteins found in the cell wall of bacteria, such as *E. coli*. These proteins will react with factors found in the bloodstream of patients, resulting in elevated body temperature. Endotoxin testing has been referred to as pyrogen testing in the past (pyrogen = high temperature) because it results in a high temperature in humans and animals. Although this might not be fatal, it could exacerbate a pre-existing condition. Requirements for endotoxin testing are set forth in *USP* Chapter <85>.[5] The endotoxin testing method uses the Limulus Amebocyte Lysate (LAL) test. The LAL test identifies and quantitates for the presence of endotoxin reactions using amebocytes extracted from the horseshoe crab.

Two types of endotoxin tests are available: (1) the gel-clot technique and (2) the photometric technique. The former involves gel-clot formation with LAL. The latter involves a turbidimetric method to test turbidity and a chromogenic method to measure color change.

Endotoxins cannot be easily filtered or removed from finished preparations. If glassware is suspected as the source of contamination, it is possible to depyrogenate glassware by heating it in an oven at 250ºC for 30 minutes (*USP* Chapter <85>).[5] Items may also be rinsed with endotoxin-free water, but this is not guaranteed to be successful. Acceptable endotoxin levels are relative to the use of the preparation. Endotoxin testing for parenteral drugs can mainly be used for inhalation prepara-

Table 2. Example of Protocol for Number of Samples To Be Tested.

Quantity of Sterile Units per Batch	Number of Samples	Tests
Up to 25 units	1	1 – potency 1 – sterility 1 – endotoxin
26 to 100 units	2	1 – potency 2 – sterility 2 – endotoxin
101+ units	3	1 – potency 3 – sterility 3 – endotoxin

tions and ophthalmic preparations and oral solutions. It is very important in any situation involving a patient that may be susceptible to infection.

Concerns with endotoxin testing include the possibility of false positives; for instance, beta glucans (carboxymethylcellulose) has been known to cause false positives. Reagents are available to neutralize the reaction (for example, Glucoshield, a beta-glucan blocker, Cambrex, Chicago, Illinois). Some chemicals may interfere with the LAL reaction. A series of dilutions of the sample needs to be performed to minimize or negate their interference. This is known as inhibition/enhancement, and it should be evaluated to establish that the results are not false negative.

Number of Samples To Be Tested

There is no generally agreed-upon protocol for determining the number of articles to be tested. Each practice should develop its own protocol to make such a determination. This should be based upon variables such as type of compounded preparation, volume, risk potential to the patient, procedures and personnel.

Summary

In summary, it is important for the pharmacist who extemporaneously compounds to ensure the strength, quality, identity and purity of compounded preparations. An outside analytical laboratory can assist by providing quality control and quality assurance. Quality-control testing should include, but is not limited to, sterility and endotoxin or pyrogen tests to provide a microbial-free preparation. The key to quality control is to develop a protocol that is appropriate and logical and well thought out for each compounding practice. An example of such a protocol is shown in Table 2. There have been reports of tragedies resulting from a lack of quality control in the compounding pharmacy. There is no doubt that some of these could have been avoided if the pharmacy had taken a more proactive role in quality control and assurance. Performing microbial testing such as sterility, fungal and endotoxin testing on compounded preparations will assist in achieving compliance with *USP* Chapter (797>.

References

1. United States Pharmacopeial Convention, Inc. *United States Pharmacopeia 27-National Formulary 22*. Rockville, MD: US Pharmacopeial Convention, Inc.; 2004: 2350–2370.
2. Newton DW, Trissel LA. A primer on *USP* Chapter <797> "Pharmaceutical Compounding—Sterile Preparations" and USP Process for Drug and Practice Standards. *IJPC* 2004; 8(4): 251–263.
3. Rahe H. Overview of *USP* Chapter <797> "Pharmaceutical Compounding—Sterile Preparations": The potential impact for compounding pharmacies. *IJPC* 2004; 8(2): 89–94.
4. United States Pharmacopeial Convention, Inc. *United States Pharmacopeia 27-National Formulary 22*. Rockville, MD: US Pharmacopeial Convention, Inc.; 2004; 2157–2162.
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