



# QUALITY-CONTROL ANALYTICAL METHODS: THE QUALITY OF STERILITY TESTING

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## Abstract

Microbiology, like compounding, is a science that must be demonstrated to show that it is reliable, reproducible, and scientifically sound. Aseptic technique must become second nature to the microbiologist and the compounding pharmacist. With each new drug tested by a quality-control laboratory, there is a responsibility that everything possible is done to ensure that the test result reported is accurate and reliable. While it is recognized that the conventional sterility-testing method has inherent deficiencies, an alternative method cannot be used unless it provides equivalent assurance of detecting microbial contamination. A quality-control laboratory and compounding pharmacists should adhere to the standards of the United States Pharmacopeia when compounding and testing preparations.

It is easy to become complacent with our habits, whether those habits are good or bad. Bad medical habits, however, can lead to detrimental health conditions and untimely deaths. In 1847, when Vienna, Austria, was considered the world's leading medical center, physician Ignaz Semmelweis discovered by simple observation the cause of puerperal fever, then known as childbed fever. Semmelweis implemented a solution, and, in 3 months, the death rate in the maternity ward fell from 18% to 1%. Semmelweis' solution was simple—he ordered doctors and medical students to wash their hands. He deter-

mined that a doctor going from patient to patient without handwashing could carry and spread puerperal fever, which today is known to be caused by *Streptococcus pyogenes*. Although his findings were published and even duplicated, nobody listened. It would be many years and thousands more deaths before handwashing became accepted clinical practice.

Those involved in quality control are also subject to complacency. Quality-control methods often are accepted because "that's the way it has always been done." This is an especially dangerous attitude if the test result is biased or based on the

conditions set forth, or if a better method is overlooked. A quality-control laboratory has a great responsibility to not only adhere to the guidelines, but to continually examine its employees and methods, and should refer to the literature critical to the field involved; microbiological testing is no exception.

Microbial testing in pharmaceutical compounding should be examined in two parts: (1) process validation and (2) end-preparation sterility testing. *United States Pharmacopeia (USP) Chapter <797>* outlines process validation in the sections titled "Personnel Training and Evaluation in Aseptic Manipulation Skills" and "Environmental Quality and Control."<sup>1</sup> End-preparation sterility testing falls under the "Finished Preparation Release Checks and Tests" section.<sup>1</sup> Each section is important, but the focus here is only on sterility testing.

## History

Since the inauguration of sterility testing in 1936, great improvements have been made in our ability to detect microbial contamination in pharmaceutical compounds. When sterility testing was

introduced, in *USP-National Formulary (NF) 11*, it was recommended only for liquid preparations and required only a 7-day incubation period and one type of culture medium. Over the 70 years since *USP-NF 11* was published, *USP* sterility testing methods and the media recommended have been revised frequently in ongoing attempts to improve the detection of microbiological contamination. Today, *USP* Chapter <71> requires the use of two culture media, Soybean-Casein Digest Medium (SCDM) and Fluid Thioglycollate Medium (FTM), and a 14-day incubation period.<sup>1</sup>

### Sterility Testing

*USP* Chapter <71> states that "...sterility testing is a very exacting procedure, where asepsis of the procedure must be ensured for a correct interpretation of results..."<sup>1</sup> The *USP* also states that alternative methods may be utilized as long as they are validated and "will yield results equivalent to, or better than, the results generated by the conventional method."<sup>1</sup>

Although the compendial sterility testing is the most widely accepted method, it has inherent limitations.<sup>2-6</sup> In a review published in the *Pharmacopeial Forum*, the United States Pharmacopeial (*USP*) Expert Committee responsible for *USP* Chapter <71> Sterility Testing examined the media and incubation conditions recommended for compendial sterility testing.<sup>4</sup> Through this review, deficiencies were recognized and recommendations made, some of which are discussed in this article.

### Limitations to Compendial Sterility Testing

#### Sample Size

One of the recognized limitations of sterility testing is sample size.<sup>1,5,6</sup> *USP* Chapter <1211> notes 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."<sup>1</sup> Since the absolute sterility of a preparation lot cannot be demonstrated without complete destruction of every

article, and there are other limitations of the sterility testing itself, everything possible must be done to ensure that the preparation is safe to dispense. This would include ensuring that the sterilization process and aseptic processing procedures are validated and personnel properly trained and qualified for compounded sterile preparations. The compounding pharmacy should verify that its preparations are free of microbial contamination.

### Testing Conditions

It is vital that a detection procedure be in place in case contamination occurs. Currently, *USP* Chapter <71> states that SCDM and FTM should be incubated at  $22.5^\circ \pm 2.5^\circ\text{C}$  and  $32.5^\circ \pm 2.5^\circ\text{C}$ , respectively.<sup>1</sup> SCDM is used for promoting the growth of aerobic bacteria and fungi, while FTM is used primarily for anaerobes but will grow some aerobes. Both the primary and secondary literature have shown,

however, that these are less than optimal growth conditions for many bacteria and fungi.<sup>2,4,6-8</sup>

While the recommended temperature of  $22.5^\circ \pm 2.5^\circ\text{C}$  for SCDM may be aimed at detecting environmental contamination, it has been well documented that most clinically significant bacteria grow at temperatures between  $25^\circ\text{C}$  and  $40^\circ\text{C}$  and fungi at temperatures between  $25^\circ\text{C}$  and  $30^\circ\text{C}$ .<sup>7,8</sup> The manufacturers of the fungal growth medium recommended in the *USP* recommends incubation temperatures in the range from  $25^\circ\text{C}$  to  $30^\circ\text{C}$ .<sup>9</sup> One of the recommendations made by the *USP* Expert Committee for Sterility Testing was to increase the incubation temperature to  $27.5^\circ \pm 2.5^\circ\text{C}$  to optimize the recovery of bacteria, yeasts, and molds—a change that has yet to occur.<sup>4</sup>

A comprehensive study of the growth-promoting characteristics of seven different media examined 88 different



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strains of bacteria, 38 strains of yeast, and 54 strains of mold.<sup>2</sup> This study concluded that SCDM and FTM were less effective than dithionite-thioglycollate broth (HS-T) at growing both aerobic and anaerobic bacteria when incubated at 32°C. It was also shown that SCDM was less effective in promoting growth of fungi and yeasts than four other media when incubated at 26°C for 10 days. The four media were Sabouraud Liquid Medium, Sabouraud Dextrose Agar, Peptone Liver Digest Broth, and Peptone Liver Digest Agar.

*USP* Chapter <61> Microbial Limit Tests states that Sabouraud Dextrose Agar Medium or Potato Dextrose Agar Medium should be used instead of SCDM for performing total combined counts of molds and yeasts.<sup>1</sup>

While the addition of another growth-promoting medium adds more cost to the sterility-testing process, a risk assessment should be performed. Special consider-

ation should be given to the environmental quality of the compounding facility, taking into account humidity levels, temperature, and the type of organisms that have been isolated from the compounding area. If these variables are unknown, an inexpensive thermometer and hygrometer combination device can be purchased, as can pre-made media for testing for organisms in the environment. Using media specific for bacteria and fungi, the compounding area should be sampled, using contact plates, settling plates, or an air sampler to determine the type and extent of contamination that may exist. *USP* Chapter <797> offers information for key areas to examine and how often they should be tested.

Another area of discussion is the methods employed for sterility testing, which are (1) membrane filtration and (2) direct inoculation. Some advantages of the membrane filtration method are

the ability to test more sample using less medium, fewer false positives, and greater sensitivity than the direct inoculation technique.<sup>4</sup> These advantages also could be cited for direct inoculation if a validated alternative method is used with the right incubation conditions and appropriate growth medium.

The 7-day incubation period was at one time thought sufficient for preparations tested by membrane filtration because it was theorized that the antimicrobial properties would be removed through the filtering process and growth would occur earlier. This requirement was changed to a 14-day incubation period in *USP-NF 24*, the same as was prescribed for direct inoculation. This change was implemented on the recommendation of the *USP* following emergence of a growing body of evidence that an unacceptable amount of growth was occurring after the 7-day incubation period.<sup>3,4</sup> It was also demonstrated that, for the preparations tested, there was no significant difference in rates of detection of positives between the direct inoculation method and the membrane filtration method after 14 days of incubation.<sup>3</sup>

Another study concluded that solid media were effective growth-promotion materials, and, in some cases, growth was observed 1 to 2 days earlier in solid media than in broth.<sup>10</sup> This 1- to 2-day lead time can be crucial in identifying and correcting contamination problems to minimize compounding down time. In the case of hospital compounding, where many times compounds have to be made and dispensed the same day, the earlier a breakdown in the aseptic process is detected, the quicker the medical staff can notify the patients affected and begin monitoring the patients for adverse events.

### Looking Ahead at Sterility Testing The Future

Many times quality-control laboratories view alternative methods as “taboo.” This may be warranted if the methods haven’t been validated against the standard of *USP* Chapter <71>. Furthermore, while the industry may never change the 14-day sterility testing incubation requirement, there

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still must be an effort to improve early detection of microbial contamination. Rapid microbiological analysis is still relatively new in the pharmaceutical industry, and there are several new products and methodologies, using more sensitive biological markers, that could be employed in the compounding setting.<sup>6</sup>

The U.S. Food and Drug Administration encourages the pharmaceutical industry to use new technologies that will allow for real-time quality assurance. With the advent of new technologies and methodologies, the opportunity for preparations to be released in days instead of weeks has been recognized.<sup>8</sup> A more comprehensive overview of the pros and cons of the new methods on the horizon and an appropriate validation protocol is available from articles written by Moldenhauer and Sutton,<sup>6</sup> Riley,<sup>11</sup> and Sutton.<sup>12</sup>

## Conclusion

Microbiology, like compounding, is a science that must be demonstrated to show that it is reliable, reproducible, and scientifically sound. Aseptic technique must become second nature to the microbiologist and compounder and, as they teach in graduate school, those involved in the sciences must begin to “think like the bugs.” Care must be taken during the compounding process to ensure that the preparation being made is of the highest quality, and microbiological testing is no exception. On a daily basis, quality-control laboratories are on the front line of testing newly formulated preparations. With each new

drug tested, there is a great responsibility that everything possible is done to ensure that the test result reported is accurate and reliable. While it is recognized that the conventional sterility-testing method has inherent deficiencies, an alternative method cannot be used unless it provides equivalent assurance of detecting microbial contamination.

Too many times, quality-control laboratories get caught up in the old adage “if it ain’t broke, don’t fix it.” Often, it is not just that something is “broken,” but that it can be improved. Imagine if Dr. Semmelweis hadn’t decided to make a difference; it would have been easier for him to keep going along like everything was fine. Quality-control laboratories should think the same way and want to make a difference.

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