

Importance of Background Microbial Levels in the Manufacture and Testing of Sterile Products

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The sterility testing of samples from an aseptic process may be considered an **entirely separate aseptic process that is subject to the same types** of adventitious contamination as the aseptic process itself.

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Sterile products produced in staffed cleanrooms are subject to microbial contamination from the environment in which the process is carried out. The process may be adversely affected by the presence of microorganisms termed “adventitious,” (*i.e.*, contamination incidental to the process) but also by contamination that is an unavoidable consequence of that process. Furthermore, there is no direct method to establish the source of contamination in aseptic processing environments. Contamination may be derived from the process, materials, equipment, operators, or the production environment, but could just as easily be introduced during sampling or the testing of samples (1). The sterility testing of samples from an aseptic process may be considered an entirely separate aseptic process, subject to the same types of adventitious contamination as the aseptic process itself. Nonetheless, contaminants found in samples taken from the production environment, whether for sterility or environmental monitoring, are routinely associated solely with the production environment and not considered adventitious contaminants introduced during the testing. As long as the background contamination rate in the sterility and environmental testing environments is sufficiently low, it can be assumed that all contamination is derived from the production area.

Pre-isolator aseptic operation

Using a cleanroom to perform sterility testing was the industry standard for many years because it was assumed that the “false positive” rate in sterility testing was low enough to preclude loss of product caused by background contamination. Evaluating the methods and environment in which samples were analyzed was not seen as important. The potential for sampling- or laboratory-induced contamination was considered low enough to be acceptable because the production environment was substantially less capable in that era, and experts at the time recognized that the aseptic environment could never be considered sterile (2). The overall situation, depicted in Figure 1, with the vertical scale representing the background microbial count in which the activity (aseptic processing, sterility testing, or environmental sample workup) is taking place. The relative heights of the columns relate to the potential for in-

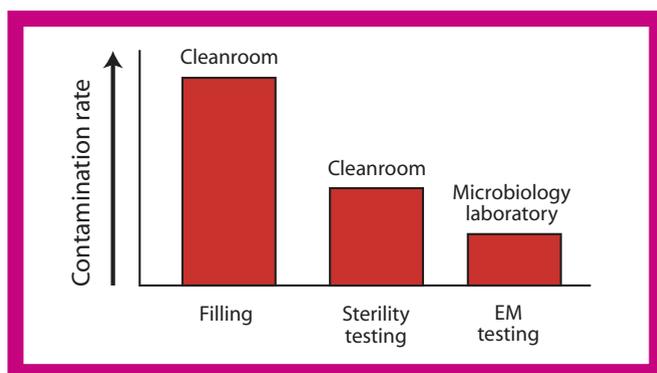


Figure 1: Relative contamination rates during the period when filling and sterility testing were conducted in cleanrooms (through ~1985).

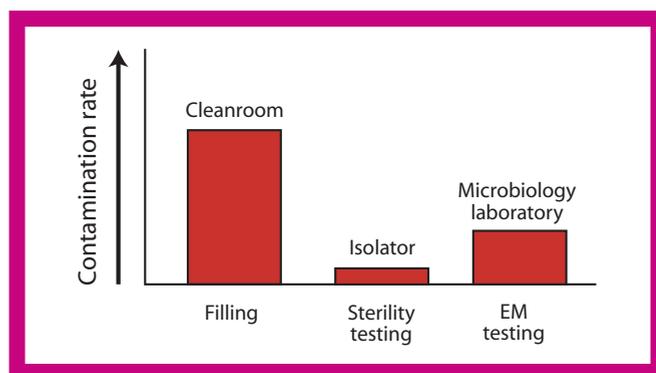


Figure 2: Relative contamination rates when sterility testing is conducted in an isolator.

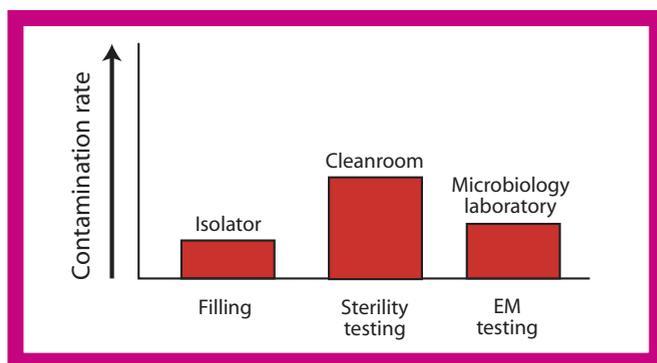


Figure 3: Relative contamination rates when filling is conducted in an isolator but testing is performed in a cleanroom.

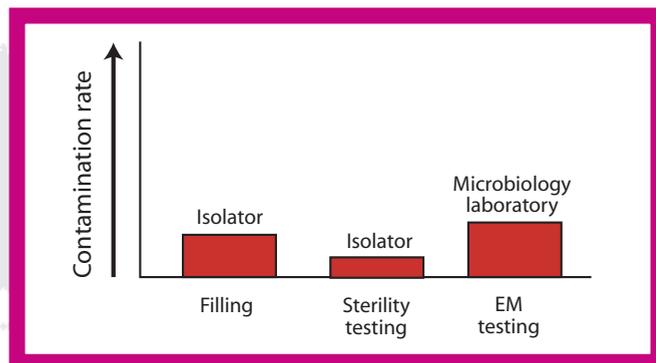


Figure 4: Relative contamination rates when isolators are used for both filling and testing.

duced contamination from the environment.

The example in Figure 1 applies to the time period from the inception of staffed cleanrooms for use in aseptic processing until the mid-1980s. The emphasis placed on validating sterile products that started in the mid-1970s began to have an impact approximately 10 years later. As the capabilities of staffed cleanrooms improved and greater attention was paid to all aspects of sterile manufacturing, the limitations of the staffed cleanroom for sterility testing became evident. The US Food and Drug Administration addressed these limitations by tightening its guidelines regarding the execution of testing, making it all but impossible for firms to retest products that failed the initial sterility test (3). It was understood that the sterility test itself was flawed, but companies adhered to US Food and Drug Administration expectations to ensure patient safety.

The advent of isolator-based sterility testing

One of the consequences of abandoning the sterility retest was the rapid adoption of isolators for sterility testing, which all but eliminated the background contamination potential during sterility testing. Thus the risk of false positives was practically eliminated (see Figure 2).

The situation depicted in Figure 2 is commonplace today, as the cleanroom manufacturing of aseptically filled products is still predominant. Because the cleanroom is merely aseptic, background contamination from the environmental testing laboratory can still be ignored—any detected contamination is assumed to be from the manufacturing process itself. Note that

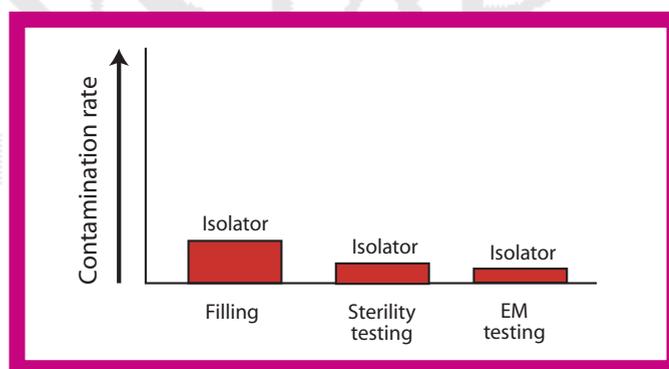


Figure 5: Relative contamination rates when isolators are used for all activities.

the contamination rate for staffed cleanrooms has been reduced relative to Figure 1, thereby reflecting the improvements to aseptic operations over the intervening years (4).

Isolator-based aseptic processing

There have been some missteps in introducing isolation technology in the industry. Figure 3 depicts one such error, in which an isolator is used during the aseptic process but testing is performed in a cleanroom. The difficulty with this combination is that the background contamination level in the test environment is such that a sterile product (produced in the more-capable production isolator) may be considered nonsterile because of contamination arising from the less-capable staffed cleanroom in which sterility testing was conducted. Firms that

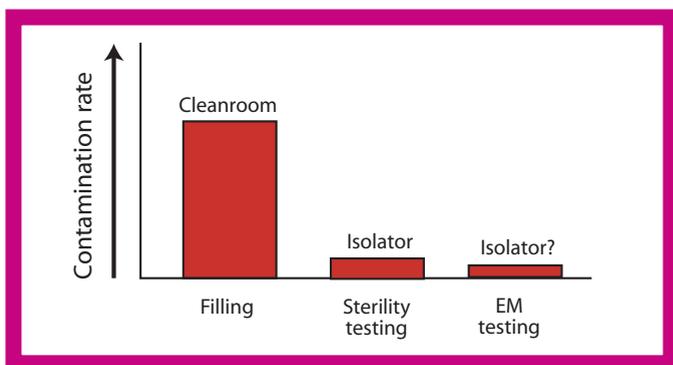


Figure 6: Relative contamination rates when using an isolator for environmental monitoring to meet FDA's guidance.

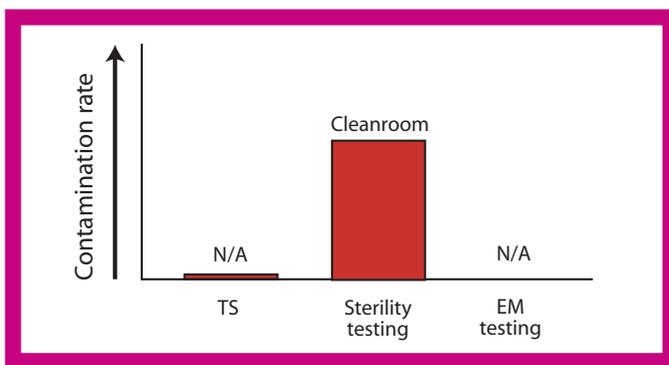


Figure 7: Relative contamination rates resulting from improper testing of terminally sterilized products.

progressed in this manner soon realized that the sterility test should be conducted in an isolator system to avoid the potential for a high rate of false positives.

The preferred approach for implementing production isolators is for both the aseptic process and the sterility test to be performed in isolators, as depicted in Figure 4 (5). This approach represents the growing trend toward isolator-based operations for all aseptic operations. Note that the envi-

ronmental monitoring workup for both the production and sterility test environments has remained unchanged because many firms have not introduced isolators for that purpose.

What has been largely ignored during the industry's transition to isolators has been the treatment of environmental monitoring. According to FDA, internal isolator surfaces must be sterile at all times. Any surface or airborne contamination detected

in an isolator may be sufficient reason to reject the materials produced while that contamination was present (6). The environmental monitoring samples from the isolator have in part assumed the role of the sterility test as the arbiter of the sterility of materials produced in the isolator. The natural consequence of this method is to use isolators for every aspect of production, sterility testing, and environmental monitoring. Although this approach may be considered prohibitively expensive to some, it has already been adopted by firms that have recognized that environmental monitoring has become this era's sterility test. These firms agree that false positives in environmental testing cannot be tolerated any more than they could in sterility testing. These methods represent a return to the relative contamination risk rates that prevailed before the introduction of isolators for any activity. Because of their complexity, production processes must always be considered to have a greater potential for adventitious contamination than either sterility testing or environmental monitoring performed in a comparable environment.

Increased emphasis on environmental monitoring

For firms that have not yet adopted isolation technology for aseptic processing, FDA's 2004 aseptic guidance on the subject introduces new complications (6). A clear expectation exists that environmental samples from critical surfaces in the aseptic manufacturing environment must be devoid of contamination. As a result, firms may wish to convert to isolator-supported environmental monitoring to eliminate action against products because of adventi-

tious contamination introduced during environmental testing (see the contamination risk levels depicted in Figure 6). This approach might also be appropriate for other highly capable aseptic-production systems such as blow-fill-seal and form-fill-seal.

Although isolators may not be the perfect answer to every aseptic processing situation, they are rapidly becoming the technology of choice for a variety of applications (2). The full potential for these systems can be realized if their application is carefully considered. The recognition that environmental monitoring may represent the last loophole in the system could lead to alternative methods (*i.e.*, conducting environmental monitoring testing in an isolator), thus resulting in reliable operation, less risk to the patient, and more rapid decision making.

Cautions in testing terminally sterilize products

More than a decade of experience has shown me that many firms have erred in the means by which they control their sterile production operations. Figure 7 depicts an example of the contamination risk levels resulting from a poor combination of production and testing approaches. Although terminal sterilization is not perfect, the potential for testing-induced microbial contamination must be deemed substantially higher than any potential for microbial survival of terminal sterilization. Parametric release may not be widespread, but the added assurance that the sterility test affords to a terminal sterilization process is perhaps comparable to the addition of belt and suspenders to an elastic waistband on a pair of pants. Terminally sterilized products should *never* be tested for sterility in an ordinary cleanroom. As long as parametric release is considered a possibility for only the largest and most capable firms, the rest of the industry should at least avoid the mistake of relying on outdated cleanroom sterility testing for the release of their materials. The logical way to test terminally sterilized products is using an isolator,

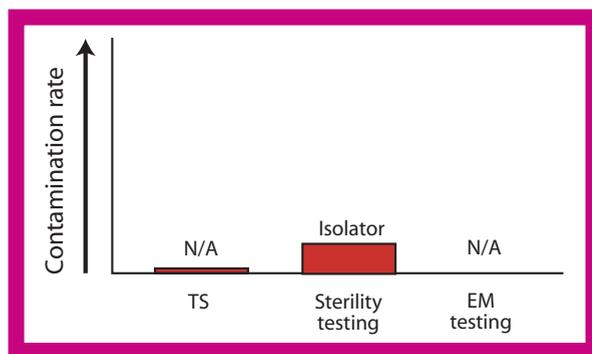


Figure 8: Relative contamination rates resulting from proper testing of terminally sterilized products.

which will produce the relative contamination risk rates shown in Figure 8.

Conclusion

This paper has reviewed the recent history of aseptic processing, sterility testing, and environmental monitoring with the goal of increasing awareness of the possible pitfalls that can be encountered. As technology changes, it behooves companies to be aware of the full implications of technological advancement to be sure that measurement tools are appropriate matches with the production methodologies.

References

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