All drug products purported to be sterile must undergo sterility testing of the final product, which is a mandatory release test required by cGMP and described in the European Pharmacopoeia (Ph. Eur.) and the United States Pharmacopeia (USP) (1, 2). Microbiological contamination can lead to recalls, compromise patient safety, and damage a manufacturer’s reputation.

In many parts of the world, regulatory and industry guidelines encourage the validation and adoption of rapid microbiology methods. The Ph. Eur., for instance, has created a dedicated reference (5.1.6) entitled “Alternative Methods for Control of Microbiological Quality,” which provides guidelines for rapid methods including rapid sterility testing (3). USP did the same with chapter <1223> “Validation of Alternative Microbiological Methods” (4). Similarly, the Parenteral Drug Association (PDA) includes rapid sterility testing in its Technical Report 33, Evaluation, Validation and Implementation of Alternative and Rapid Microbiological Methods (5).

While guidelines are evolving and the need for a faster approach to sterility testing is well recognized, few rapid microbial systems have been validated, implemented, and approved by regulatory authorities. This lack of explicit approval has slowed general acceptance and consequently adoption. This article describes the evaluation and validation process of a rapid sterility testing method that was designed to deliver results in five days rather than 14.

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Sterility Testing

Traditional methods
The two traditional methods for sterility testing described by the *Ph. Eur.* and *USP* (1, 2) are membrane filtration and direct inoculation. Membrane filtration should be used whenever the product is filterable, and direct inoculation when the product cannot be filtered. Both methods are based on the capacity of contaminants to grow, proliferate, and become visible in an incubation medium.

They require two liquid culture media: fluid soy-bean casein digest medium (trypticase soy broth or TSB) and thioglycollate medium (FTM), which are meant to allow recovery of all types of microorganisms normally present in a pharmaceutical environment, including aerobic and anaerobic bacteria, yeasts, and molds.

Among the major disadvantages of traditional sterility testing methods is the subjectivity of the visual examination of the results. Because turbidity must be visually verified by laboratory personnel, the methods are subject to increased risk of human error.

Another disadvantage is that traditional sterility testing methods require at least 14 days to complete. During this time, companies incur costs to hold their products in storage until sterility is proven. Additionally, in case of test failure (i.e., growth), a corrective action to the process may not be performed quickly enough, thus compromising the quality of future product batches.

Identifying a new method
As a contract research organization that offers microbiological testing, Confarma generally uses membrane filtration and direct inoculation to perform sterility testing. However, the company sought to provide an alternative method with faster results to enable its clients to release products sooner and, in the case of a non-sterile product, to start an investigation earlier.

The Confarma team identified four requirements for the new system:

- The alternative method had to be similar to the traditional test, to facilitate data interpretation and method validation.
- The test had to be performed in an isolator to reduce the risk of false positives.
- In case of a positive result (contamination), the method had to be compatible with available identification methods for further investigation.
- For ensured quality of performance, the system had to have been studied by regulatory authorities previous to Confarma implementation.

Confarma decided to work with the Milliflex Rapid system (EMD Millipore), which met these criteria. Researched literature included articles written by Novartis (Basel, Switzerland) describing the validity of the system application and its regulatory acceptance from different authorities including the European Medicines Agency and...
FDA (6, 7, 8). In an independent study, the FDA Center for Biologics Evaluation and Research (CBER) confirmed the method to be acceptable as an alternate sterility method in comparison to other rapid systems (9).

Implementing a new approach in an existing laboratory can be difficult. There is a learning curve associated with new equipment, and the laboratory may have to be redesigned to accommodate the new approach.

When working with different requirements (Ph. Eur./USP/PDA), there is varying information needed to comply with each; this necessitates additional research and organization to ensure compliance across all regulations. Additionally, alternative method validation demands that data be generated to verify that results meet specifications, and to show that the method is equivalent or superior to the traditional method.

**Validation**

Because Confarma has developed and validated several rapid methods in microbiology, including mycoplasma detection by quantitative polymerase chain reaction (qPCR), a set of best practices were already in place before starting the process. These steps included:

- thorough study of the regulations governing the method, to ensure compliance
- discussion with regulatory authorities, to ensure that all parties agreed on the proper regulations and procedures
- evaluation of other needs specific to the method
- a multidisciplinary team to support the validation process.

**Figure 1: Stages of the validation process. IQ is qualification of installation. OQ is operational qualification. PQ is performance qualification, which has multiple steps. Confarma is currently in stages PQ2-1 and 2.**

- **IQ**
  - Qualification of installation
  - Done by EMD Millipore
  - Finished and fulfilled

- **OQ**
  - Operational qualification
  - Done by EMD Millipore
  - Finished and fulfilled

- **PQ**
  - Performance qualification
  - Done by Confarma in several steps
  - First part finished
  - Second part in progress

- **PQ 1**
  - Validation with a model with microorganisms and without product
  - Done by Confarma
  - Finished and fulfilled

- **PQ 2-1**
  - Suitability testing: product versus alternative method
  - Done by Confarma
  - Ordered by the customer

- **PQ 2-2**
  - Comparability testing with both methods on one product
  - Done by Confarma
  - After a successful PQ 2-1
Sterility Testing

The team was set up with the following roles and responsibilities:

- microbiologist, to be responsible for validation design, issuing the protocol and results, and overall project management and decision making
- technician, to perform manipulations and technical review of the protocol and results
- statistician, to analyze results, provide statistical data, and recommend a conclusion
- quality assurance specialist, to review the protocol and results to verify that the regulatory requirements were satisfied, and to be responsible for approval of the final documents
- responsible pharmacist, to provide overall review and approval of the project.

Through cooperation with the technology supplier and a detailed validation protocol provided by the technology supplier, the validation process was streamlined (see Figure 1).

The proposal for validation included three main steps:

- primary validation (PQ0)
- performance qualification (PQ1)
- validation for the intended use with suitability and equivalence testing (PQ2).

During primary validation, EMD Millipore characterized and validated the system and the principle of detection according to regulatory guidelines using a model system and a panel of test microorganisms.

Once the method has been characterized by the supplier, the principle of detection does not need to be verified by each user. Confarma followed the validation proposal of EMD Millipore and started the validation steps at PQ1. PQ1 was performed by Confarma with a neutral matrix to verify that the conditions of the laboratory could satisfy the criteria described and validated by EMD Millipore during PQ0. Once PQ1 was verified, PQ2 was ini-

Figure 2: The Milliflex Rapid method (RM) achieved a superior limit of detection (LOD) to the compendial method (CM) for Propionibacterium acnes (P. acnes) and Micrococcus luteus (not shown) and LOD equivalency for other microorganisms tested, such as Candida albicans (C. albicans).
tiated by Confarma to assess the suitability of the method on products.

Confarma is now in the final stages of validation of the Milliflex Rapid method. The method will then proceed through approvals with the relevant authorities depending on the product being tested.

Validation demonstrated that the rapid method provides results in five days compared to 14, and it is superior to the traditional method according to the Ph. Eur. 5.1.6 and USP <1223> (3, 4). For example, the rapid method achieved a better limit of detection (LOD) for the microorganisms Propionibacterium acnes and Micrococcus luteus and exhibited LOD equivalency for the other microorganisms tested (see Figure 2). When testing for accuracy and precision, the rapid method performed better than the traditional method for Micrococcus luteus and was equivalent for the other microorganisms tested.

In addition, the Milliflex Rapid method is similar to the traditional method, thus the testing workflow was not disrupted. Preparation and disinfection procedures are the same, and both methods are performed using an isolator and based on membrane filtration to measure growth at three incubation conditions (i.e., anaerobic, aerobic for yeast and molds, and aerobic for bacteria). The maintenance of the existing workflow enables an easier transition to a new method.

**Conclusion**

Sterility testing of final pharmaceutical products is crucial to ensure consumer safety. However, traditional methods for sterility testing require at least 14 days to obtain results. The resulting lengthy product storage can delay time to market and increase costs for companies.

If performed using best practices and guidance from appropriate regulations, validating a new method is not an arduous process. Using similar conditions to the traditional membrane-filtration method allows for easier method equivalence validation and data interpretation. Validation of the Milliflex Rapid method for sterility testing demonstrated that it is a viable alternative to traditional sterility testing and reduces time to result from 14 to five days.

**References**