QUALITY CONTROL:

Rapid Sterility Testing for Compounding Pharmacies

ABSTRACT

Rapid sterility testing offers numerous benefits compared to a traditional sterility test, most notably shortened incubation times and reduced subjectivity in results analysis. The wait for traditional sterility results introduces unnecessary risks into the production process in the event of contamination, additional storage requirements, merchandise hold times, and delays to market. Various available technologies deliver quality-control results in just a number of days or hours, allowing stakeholders to quickly confirm the presence or absence of microbial contamination.

Andrew Taylor, MS

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The author is the Microbiology Lab Supervisor for ARL Bio Pharma, Oklahoma City, Oklahoma.

Rapid sterility testing is part of a larger group of methods termed "rapid microbiological methods" (RMMs). This term generally includes rapid sterility test methods but is more broad where it also includes bioburden testing, rapid environmental monitoring, etc. Rapid sterility testing offers an alternative to traditional sterility testing such as *United States Pharmacopeia* (*USP*) Chapter <71>, as well as the European and Japanese compendial equivalents, which allows for shortened test incubation times. Where *USP* <71> requires between 14 days to 18 days of incubation prior to a final test result,[1] a rapid sterility test result can be generated in as little as a few hours. To reduce, or in some cases eliminate, the incubation times required, rapid sterility systems use modern technology and automation to detect the presence of microbial contamination. Since timely microbiological data are often critical, not only for product release, but also for continuous process monitoring and control, rapid sterility testing is becoming more prevalent.

BENEFITS OF RAPID STERILITY METHODS

Rapid sterility testing offers numerous benefits compared to a traditional sterility test, most notably shortened test times. The wait for a traditional sterility test result introduces risk into the production process in the event of contamination, additional storage requirements, merchandise hold times, and delays to market. Products with short shelf lives are prime candidates for testing using rapid sterility methods. Faster test times allow for improved efficiency across many facets of a production cycle, including storage and distribution. This can result in reduced waste of products by extending time between the completion of the sterility test and the beyond-use date (BUD). These efficiency gains create further benefits by allowing pharmacies to respond more rapidly to patient needs.

In addition to efficiency and production gains, an extremely noteworthy benefit to shortened sterility test times is that it offers the ability to

perform investigations in a more timely manner. Rather than waiting 14 days to 18 days for a traditional test result, an "under investigation" or "not sterile" result closer to the date of production allows for a more effective investigation, as well as the ability to implement appropriate corrective actions sooner. With a faster response, comes a faster recovery following any contamination event.

The benefits of rapid microbial detection extend beyond just a faster time to result for quality-control testing. Users of many systems also gain the ability to address and meet data integrity expectations and requirements from regulatory bodies. With many of the available technologies, the implementation of a rapid sterility method replaces the subjectivity of traditional visual confirmation with an automated, reagent-based assay, controlled by an instrument. Results are generated and reported through the software, avoiding transcription and interpretation errors potentially caused by an analyst recording and reporting handwritten results. Instrument usage logs can also be generated, which can aid in meeting audit trail requirements.

AVAILABLE RAPID STERILITY TECHNOLOGIES

Many RMM technologies have been developed over the last 20 years to 30 years. Early on, many of these were marketed heavily in the clinical sector. However, some have specific pharmaceutical applications, whereas others have been developed solely for the pharmaceutical industry.

There are many rapid sterility platforms, all of which utilize varying microbial detection methods to shorten or eliminate the incubation step required in traditional sterility testing. Generally, there are two main categories of rapid sterility methodologies, growth based and non-growth based.

Growth-based Methods

Growth-based methods require an enrichment (or growth) step before microorganisms can be detected. That is especially true in samples containing low levels of contamination. These methods differ from conventional culture methods in that they rely on the detection of biochemical or physiological growth indicators rather than visible (or macroscopic) growth.

Non-growth-based Methods

Non-growth-based rapid sterility methods, on the other hand, do not rely on microbial growth to detect contamination, but instead use various cell labelling techniques to detect and quantify viable microorganisms. This approach has the potential to detect a wide range of organisms, within only a few minutes.

The differences between available rapid sterility methods are numerous, and ultimately a pharmacy must assess the strengths and weaknesses of the competing systems to decide which best meets their needs.

FACTORS TO CONSIDER WHEN IMPLEMENTING RAPID STERILITY TESTING

First and foremost, it is important to always remember that patient safety is best served by detecting microbial contamination prior to a product's administration. In order to decide if rapid sterility testing meets a company's needs, and further what methodology is the best fit, several factors play into this risk-based decision. Table 1 lists some of these factors which should be taken into consideration.

VALIDATION OF RAPID STERILITY METHODS

Perhaps the most important facet of performing a rapid sterility test is the completion of a thorough method validation. Platforms marketed for rapid sterility testing have undergone full validations by their respective vendors, as well as numerous labs. Product validations using these systems have been approved by regulatory agencies worldwide, including the U.S. Food and Drug Administration (FDA). It is important to note that the FDA has not blanketly accepted any rapid technologies as

a whole. They are, however, open to reviewing method validations, which may then be approved on a case-by-case basis. Many state pharmacy boards approve of the use of rapid sterility test methods, but, before moving forward with this testing, it is prudent to confirm with the appropriate state board. It is also important to be sure the method has been validated correctly.

TABLE 1.

FACTORS TO CONSIDER WHEN IMPLEMENTING RAPID STERILITY TESTING.

Time to Result	Methods which do not require a growth step offer the fastest turnaround time. If time to result is the most important factor, looking into one of these may be the best option.
Specificity and Sensitivity	Can the method detect the appropriate range of organisms so there is confidence in releasing a product based on a "sterile" result?
Sample Size Requirements	Often this is still based on guidance from *United States Pharmacopeia* Chapter <71>, Tables 2 and 3, but it may be possible to develop a justification for reduced sampling.
Specific Product Attributes	Many compounded sterile products and cell therapy products, due to their short beyond-use dating, would benefit from an overnight test or at least one that is completed within 48 hours, where other products may not require such a short time to result.
Confidence in the Result	A level of expertise is required to generate an accurate test result. When deciding to test in-house or use a contract laboratory, it is important to truly evaluate the knowledge and ability of the staff performing the test.
Sample Types	Not all available test methods can test all sample types. It is important to evaluate if the test method is compatible with one's products.

Historically, the task of implementing rapid sterility testing was considered very difficult and a barrier to entry for labs not familiar with alternative rapid methods. However, regulatory agencies have responded and have published guidance and documentation to assist with implementation. "PDA [Parenteral Drug Association] Technical report #33[2] – Evaluation, Validation and Implementation of Alternative and Rapid Microbiological Methods" and "*USP* Chapter <1223>[1] - Validation of Alternative Microbiological Methods," can serve as great starting points for the validation of any RMMs.

Table 2 was taken from *USP* <1223>[1] and is titled "The Validation Parameters by Type of Microbiological Test." The Table provides a list of essential tests to include in designing a validation protocol.

Since the *USP* <71> sterility test is a qualitative test, in that it yields a result of "growth" or "no growth," focus should be on the left column of Table 2.

Specificity

The specificity of an alternate qualitative sterility method is defined as its ability to detect a range of challenge microorganisms specific to the technology. A "range of microorganisms" can be defined several ways, but a good starting point would be for the validation of a rapid sterility method to include the 6 organisms listed in *USP* <71>. Oftentimes, depending on the specific needs, validation testing will also include the addition of various other environmental isolates, organisms identified from product failures, representative slow growing organisms, etc.

Specificity is demonstrated by using an organism challenge level that is above the limit of detection (around 100 colony-forming units [CFU] of each microorganism), where both the compendial and alternative methods are performed with the expectation that comparable recovery is shown.

Limit of Detection

The limit of detection (LOD) is the lowest number of microorganisms that can be detected, under the stated experimental conditions. To determine the LOD, suitable diluent solutions are inoculated with a serial dilution range of each challenge microorganism. The level of inoculation should be adjusted such that 50% of the diluted samples show growth in the compendial test. Commonly used CFU counts are 5 CFU per test, 0.5 CFU per test, and 0.05 CFU per test. This is done with the expectation that negative results will be observed using both test methods. Both the compendial and alternative tests are then performed using a sufficient number of replicates to allow for appropriate statistical analysis (generally a chi-square test or another appropriate approach).

Repeatability

Repeatability is the degree of consistency among individual test results when the procedure is applied repeatedly to multiple samplings of the same suspension of microorganisms. It then also uses different suspensions across the range of the test, to ensure the results are consistent and repeatable.

Robustness

Robustness is the capacity of the method to remain unaffected by small but deliberate variations in method parameters (e.g., reagent volume, incubation time, ambient temperature). Slight variations to these parameters provide an indication of reliability of the method and equipment during normal usage. It is important to understand that a measure of robustness is not a comparison between the compendial and alternate methods; rather, it is a necessary component of validation of the alternate test so that the user understands the limits of the operating parameters of the method.

Ruggedness

Ruggedness is the degree of precision of test results obtained by the analysis of the same samples under a variety of typical test conditions (e.g., different analysts, instruments, reagent lots). The expectation is that test results obtained by testing the same samples with varying analysts, instruments, and reagent lots are consistent. It is not uncommon for the end user to rely on data supplied by a test system manufacturer for both robustness and ruggedness determinations.

Detection of Stressed/Injured Microorganisms

While *USP* <1223> provides a starting point for designing a method validation protocol, other assessments are often included in robust validation strategies. The inclusion of the detection of stressed or injured microorganisms is common, as they are more representative of potential contaminants that may be present in pharmaceutical samples. PDA technical report #33 specifically mentions that a complete method validation should include the successful detection of stressed microorganisms.[2]

This testing is performed in a similar manner to the specificity testing mentioned above, with the addition of methodologies to apply stress or injury to the validation microorganisms prior to assessment. Some methods used to accomplish this are to expose microorganisms to certain environmental conditions (e.g., ultraviolet, heat, cold, adjustments to pH), certain antimicrobial conditions (e.g., exposure to disinfectants), or to put them through sublethal sterilization conditions. After ensuring that the appropriate reduction in viability has been achieved, testing is carried out similar to previous validation tests, in that both the alternative and traditional methods are performed using a sufficient number of replicates to allow for appropriate statistical analysis.

Ultimately, the aim of a validation is to demonstrate the acceptability of the alternate procedure relative to the current microbiological practice. To do that, the laboratory must demonstrate that the new procedure is as good as or better than the current procedure in terms of the ability to detect the presence of microorganisms; this is "equivalence." The *USP* states that "Alternate methods may be used if they provide advantages in terms of accuracy, sensitivity, precision, selectivity, or adaptability to automation."[1] It also says that they must be shown to produce equivalent or better results than the referee method for any given quality attribute. Similarly, the FDA *Guidance for Industry* document *Analytical Procedures and Methods Validation: Chemistry, Manufacturing, and Controls Documentation* states that a validated alternative procedure must be shown to have performance equal to or better than the regulatory procedure.[3] When comparing the equivalence of two test procedures, statistical

TABLE 2.

VALIDATION PARAMETERS BY TYPE OF MICROBIOLOGICAL TEST.

VALIDATION PARAMETER	QUALITATIVE TESTS	QUANTITATIVE TESTS
Accuracy	No	Yes
Precision	No	Yes
Specificity	Yes	Yes
Limit of Detection	Yes	Yes
Limit of Quantification	No	Yes
Linearity	No	Yes
Operational (Dynamic) Range	No	Yes
Robustness	Yes	Yes
Repeatability	Yes	Yes
Ruggedness	Yes	Yes
Equivalency	Yes	Yes

evidence is assembled to demonstrate non-inferiority. For example, with sterility testing, equivalency may be shown if there is no statistically significant difference between the two groups of results generated when performing testing with the compendial and alternative methods.

METHOD SUITABILITY

After validation of the alternative method is complete, method suitability is required to determine if inhibitory or interfering properties are present in a drug product that may prevent the accurate detection of viable microorganisms. Method suitability testing shows that the rapid sterility test method is valid for the specific drug product and reduces the possibility of a false sterile result. Interfering properties vary between drug products, so each product must have method suitability testing performed.

CONCLUSION

USP Chapter <71> testing has been a trusted test of a product's quality for many years. Rapid sterility testing offers numerous benefits compared to a traditional sterility test, most notably shortened incubation times and reduced subjectivity in results analysis. The wait for traditional sterility results introduces unnecessary risk into the production process in the event of contamination, additional storage requirements, merchandise hold times, and delays to market. Various available technologies deliver quality-control results in just a number of days or hours, allowing stakeholders to quickly confirm the presence or absence of microbial contamination.

REFERENCES

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Address correspondence to Andrew Taylor, Microbiology Lab Supervisor, ARL Bio Pharma, 840 Research Pkwy #546, Oklahoma City, OK 73104.
E-mail: ataylor@arlok.com