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RAPID STEAM STERILIZATION BIOVALIDATION USING BIOLOGICAL INDICATORS AND THE PALLCHEK™ LUMINOMETER

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ABSTRACT

The pharmaceutical industry's growing interest in rapid microbiological methods mandates a need to clarify regulatory and technical requirements. Instrument qualification, computer validation, and microbiological performance testing all must be considered. In light of FDA's recent approval of this technology for microbial limit testing of drug products and pharmaceutical-grade waters, the author identifies points to consider in the validation of a rapid microbiological method for rapid steam sterilization biovalidation using biological indicators and the Pallchek Luminometer.

INTRODUCTION

Conventional microbiological test methods, based on 19th-century techniques, are time-consuming and labor-intensive, lack sensitivity, are subjective, and poorly validated. Recognizing these disadvantages, the food, beverage, and cosmetic industries have been using rapid microbiological methods (RMMs) for several decades. In the pharmaceutical industry, microbiological testing is seen as a bottleneck in product release, and interest in RMMs has grown considerably over the last decade. RMMs such as ATP bioluminescence, solid-phase laser cytometry, and genetic-based identification systems are being investigated for the advantages they offer in speed, sensitivity, and accuracy.

However, despite significant progress in development of RMMs for pharmaceutical product testing, their implementation by the industry has been slow and has been marked by confusion and hesitancy, in part because of concerns about regulatory acceptance, and in part because of technical barriers. Technical barriers include the complex chemical nature of the technology, unsuitable technical transfer of systems between the food, beverage, and cosmetic sectors and the pharmaceutical sector, and lack of appropriate guidance for validation and implementation. These technical barriers must be overcome if RMMs are to be established as standard techniques.

Validation and regulatory requirements for RMM technologies are beginning to emerge as a result of FDA's endorsement of RMMs as a process analytical technology (PAT), as part of its "Pharmaceutical Quality Initiative for the 21st Century." PAT is a framework for scientific, risk-managed pharmaceutical development that requires timely, in-process measurement of materials and processes to ensure the quality of final products. The PAT initiative has encouraged the implementation of new analytical technologies and has become a driver for change and innovation in the pharmaceutical industry. Microbiological testing is an important quality indicator and an essential step in confirming process control. Real-time or near-real-time microbiological data can be generated as part of manufacturing process control only through the use of RMMs. In encouraging implementation of RMMs by the pharmaceutical industry, FDA's Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM), and Center for Biological Evaluation and Research (CBER) have recognized the need for a unified approach to the review and inspection of RMMs and are engaged in collaborative efforts, both formal and informal, with respect to review and training.

The purpose of this article is to provide information with introduction of qualitative RMMs for rapid steam sterilization biovalidation using biological indicators (BIs) and the Pallchek Luminometer, addressing such aspects as instrument qualification and microbiological performance testing.

BACKGROUND

To maintain sterility, equipment surfaces that contact a sterilized drug product or sterilized container or closure surfaces must be sterile so as not to alter purity of the drug (CFR 211.63 and 211.113). Those surfaces that are in the vicinity of sterile product or container closures, but do not directly contact the product should also be rendered sterile where reasonable contamination potential exists. It is as important in aseptic processing to properly validate the processes used to sterilize such critical equipment as it is to validate processes used to sterilize the drug product and its container and closure. Moist heat and dry heat sterilization are most widely used and are the primary processes discussed in this document. It should be noted that many of the heat sterilization principles are also applicable to other sterilization methods.

Validation studies should be conducted demonstrating the efficacy of the sterilization cycle. Requalification studies should also be performed on a periodic basis. For both the validation studies and routine production, use of a specified load configuration should be documented in the batch records (FDA-CDER Draft Guidance for Industry; Sterile Drug Products Produced by Aseptic Processing).

For the various methods of sterilization, special attention should be given to the nature or type of the materials to be sterilized and the placement of BIs within the sterilization load. The D-value of the BI can vary widely depending on the material to be sterilized. Potentially difficult to reach locations within the sterilizer load or equipment train should be evaluated in initial studies (i.e., Steam-in-Place, SIP, applications). For example, filter installations in piping can cause a substantial pressure differential across the filter, resulting in a significant temperature drop on the downstream side. BIs should be placed at appropriate locations downstream of this equipment to determine if the drop in temperature affects the thermal input at these sites. Requalification and/or revalidation should continue to focus on the load areas identified as most difficult to penetrate or heat (e.g., worst-case locations of tightly wrapped or densely packed supplies, securely fastened load articles, lengthy tubing, the sterile filter apparatus, hydrophobic filters, stopper load).

Process Validation

Heat penetration studies should be performed using the established sterilizer load(s). Validation of the sterilization process with a loaded chamber demonstrates the effects of loading on thermal input to the items being sterilized, and may identify *cold spots* where there is insufficient heat to attain sterility. The placement of BIs at numerous positions in the load, including the most difficult to sterilize places, is a direct means of demonstrating the efficacy of any sterilization procedure. In general, the thermocouple (TC) is placed adjacent to the BI so as to assess the correlation between microbial lethality and thermal input. When determining which articles are most difficult to sterilize, special attention should be given to the sterilization of filters.

Ultimately, cycle specifications for such sterilization methods are based on the delivery of adequate thermal input to the slowest to heat locations. A sterility assurance level of 10^{-6} or better should be demonstrated for a sterilization process. For more information, the reader may refer to the FDA guidance entitled *Guideline for the Submission of Documentation for Sterilization Process Validation in Applications for Human and Veterinary Drug Products*.

Developing a Sterilization Process

The objective of a sterilization process is to kill the microorganisms naturally present in the product (product bioburden) or equipment (equipment bioburden). The question a D-value study answers is how the product influences the resistance of suspended organisms during a sterilization cycle. This test is typically performed using calibrated resistant spores. Spores are the most durable of microorganisms and should demonstrate the least impact from the product. Suppliers provide this service; however, this information is only one piece of the data necessary to develop a sterilization process. Ideally, once the bioburden organisms have been characterized, D_T -value studies using these organisms in the product should be performed. Product characteristics such as viscosity, crystal formation, and pH may influence the lethality during of the cycle on the organism. This entire process may not be feasible or possible depending on the circumstances. An alternate method would be to take a conservative approach and perform a D-value study with the product using an organism resistant to the sterilization process. *Geobacillus stearothermophilus* is a spore-forming organism widely recognized for monitoring a steam sterilization process, and it may be substituted for the bioburden organism in the D-value study. When this substitution occurs, it is assumed that the *G. stearothermophilus*

organisms are more resistant than the bioburden organisms or, by design, can significantly out-number the bioburden organisms. If, by chance, the bioburden organisms have a higher resistance than *G. stearothermophilus*, then a high number of *G. stearothermophilus* spores should be used. The kill time for the *G. stearothermophilus* spores would then exceed the kill time for the bioburden organisms. Table 1 illustrates this point. The length of the cycle is determined in part by the desired Sterility Assurance Level (SAL), the initial population, and resistance of the organism. For example, if product X contains 100 microorganisms/unit with a D_{121} -value of 3.0 minutes, then a 24.0 minute exposure would give a SAL of 10^{-6} .

Table 1. Sterility Assurance Level Example.

Exposure Time at 121 °C in BIER Vessel (F.)	Number of Surviving Organisms Per Unit	Sterility Assurance Level (SAL)
0 minutes	100	Non sterile
3 minutes	10	Non sterile
6 minutes	1	Non sterile
9 minutes	0.1	1 Non sterile in 10
12 minutes	0.01	1 Non sterile in 100
15 minutes	0.001	1 Non sterile in 1,000
18 minutes	0.0001	1 Non sterile in 10,000
21 minutes	0.00001	1 Non sterile in 100,000
24 minutes	0.000001	1 Non sterile in 1,000,000

Each lot of BIs will vary slightly in its population, resistance, and kill time (exposure time in which zero tested units have positive results). The BIs used to monitor a cycle must have a reported kill time equal to or less than the validated cycle time.

The SAL value can be calculated based on the BI selected. For example, a BI with a 10^6 spore challenge with the D-value equal to 1.0 minute will have to be exposed to a process equal to 12 D-values to yield a SAL of 10^{-6} . Also, the SAL value can be based on the calculated resistance of the natural bioburden with a normal D-value less than 1.0 (normally the D-value of the natural bioburden is 0.1–0.3 minute). In this case, if we want to have a process equal to 12 D-values to yield a SAL of 10^{-6} , a BI with a much lower population of spores than the number of bioburden would be selected.

Products that are quite stable to long exposure times at 121° C use the 10⁶ BI approach. Products sensitive to heat use the combination bioburden resistance and custom BI challenge to monitor the process.

The current compendial methods for enumeration and/or growth/no growth BI control generally use either a membrane filtration-based, pour-plate/spread-plate methods or direct transfer of the BI into the culture medium (generally Tryptic Soy Broth). These growth-based test methods typically take 7 to 14 days to complete, and they require visual examination and/or identification. The current USP methods are a threefold test, entailing enumeration of microorganisms (quantitative) or growth/no growth after the BIs exposure (qualitative).

It is important that health care facility sterilizers perform effectively to prevent nosocomial infections. BIs can provide sterilizer users with information on the effectiveness of sterilizer processes. FDA regulates BIs intended to monitor health care facility sterilizers as Class II medical devices, requiring pre-market notification (510(k)). This draft guidance document recommends the kind of data and information you should submit in a 510(k) for these devices

The use of comprehensive, scientifically sound criteria helps ensure the safety and effectiveness of these devices.

Guidance documents referred to in the 510(k) document and that provide information on other medical devices are available on the Internet at the Center for Devices and Radiological Health (CDRH) home page, <http://www.fda.gov/cdrh>.

A new technology for microbial determination, ATP bioluminescence, holds particular promise for pharmaceutical testing, meeting quality demands and business requirements for speed and selectivity. This technology is far superior to traditional techniques in many respects.

The ATP bioluminescence assay provides a fast and effective means of determining the presence of viable microorganisms (including spores) and their enumeration. The firefly *Photinus pyralis* produces light naturally due to a process called bioluminescence, when the enzyme luciferase catalyzes the reaction of luciferin with the nucleotide adenosine triphosphate (ATP). Thus, luciferase can be used to detect the presence of ATP (found in all living cells, including microbial cells) quickly and accurately by light production with the result is expressed in RLUs (Relative Light Units). Validation requirements for microbial limit testing with RMMs are defined in Parenteral Drug Association Technical Report 33, "Evaluation, Validation and Implementation of New Microbiological Testing Methods" (PDA 2000). The validation criteria, listed

in Table 2 for both qualitative and quantitative methods, are independent of the technological platform. The equivalence of RMMs to conventional methods can be demonstrated through a series of experiments designed to assess the validation criteria, which can be performed by the prospective user or by others (USP 2005).

Parameter	Qualitative ATP Bioluminescence
Accuracy	—
Precision	—
Specificity	✓
Equivalence	✓
Limit of Detection	✓
Limit of Quantitation	—
Linearity	—
Range	—
Repeatability	✓

Table 2. MLT Validation Criteria for Rapid Microbiological Methods.

Validation of RMMS for BIs

Because of the diversity of requirements for BI enumeration and monitoring, no single technology platform has the capacity to satisfy all testing requirements (quantitative and qualitative tests). The use of an RMM for quantitative testing on BIs (initial enumeration)

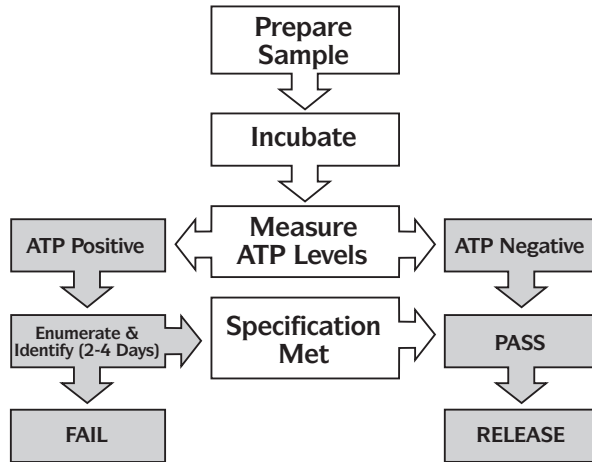
and qualitative testing on BIs after the sterilizer exposure could be used as replacement of the use of the currently approved conventional method.

For the two approaches, the RMM is utilized using two different protocols. Use of the RMM protocols allows rapid sterilizer release in a matter of hours versus days or weeks, for sterilizers that demonstrate the absence of BI growth after a sterilizer cycle. The ATP bioluminescence method could be validated for qualitative and quantitative tests. Validation and implementation are no different for RMMs than for the traditional tests.

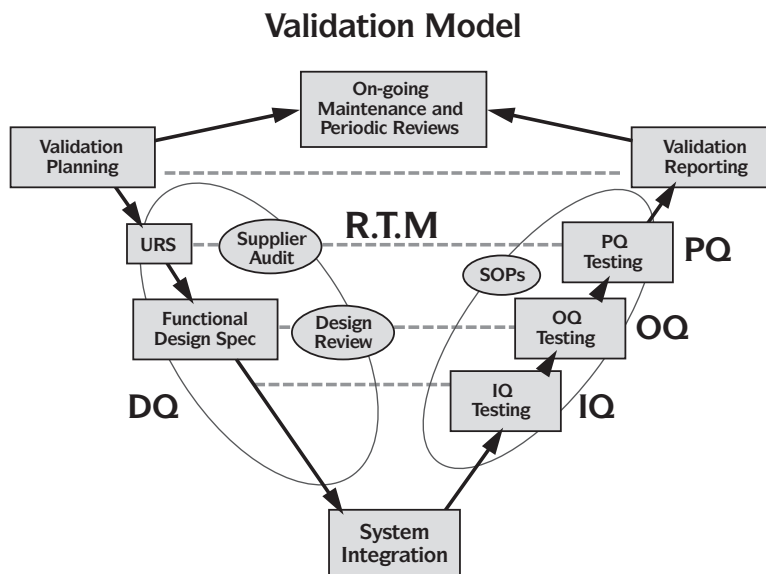
A strategy can be developed that allows for rapid release of the sterilizer when the RMM gives negative results for the presence of ATP after sterilization cycle. When the test indicates the presence of microbial ATP, the investigation can determine the numbers and types of microorganisms present and demonstrate compliance or noncompliance with the microbiological specification. Figure 1 illustrates this process.

Figure 1. Qualitative Testing Strategy for Two-tiered Testing, Using the ATP Bioluminescence Method and a Traditional Method (RLU = Relative Light Units).

ATP: 2-Tier Screen Release Criteria



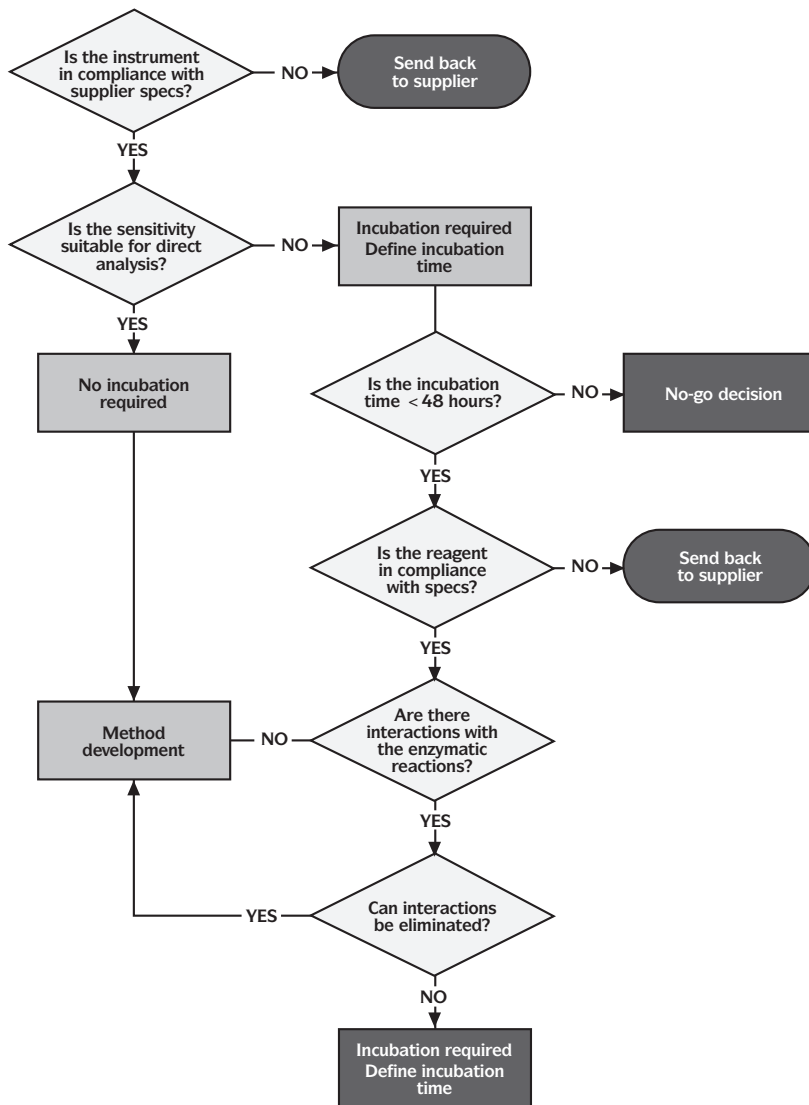
Validation of the RMMs focuses on both microbiological testing performance and technology platform qualification. A validation model could be a V-model (widely used in computer software development), modified to include microbiological performance qualification attributes (Figure 2). Other validation models exist, and use of the modified V-model in this example is not meant to suggest that it is required. However, the basic elements of design qualification (DQ), installation qualification (IQ), operational qualification (OQ), and performance qualification (PQ), as illustrated in this model, are important parts of any process for validation of a new analytical method. The modified V-model also provides a convenient way to combine equipment qualification requirements with microbiological performance evaluation.

Figure 2. The V-model for System Qualification.


Design Qualification

A validation master plan was developed that outlined the approach to RMM evaluation. This plan included both equipment and microbiological performance evaluation details such as user requirements, performance capabilities, computer hardware and software requirements, supplier audits, costs, and benefits. The flow diagram shown in Figure 3 illustrates a process for assessing the feasibility of any rapid microbiological technology, using ATP bioluminescence as an example.

Figure 3: Process for Design Qualification of Rapid Microbiological Technology, as Applied to ATP Bioluminescence.



Installation Qualification

The IQ stage verified and documented that the RMM had been supplied, installed, and adequately performed the tests as specified by the manufacturer. The IQ package included documentation of a visual inspection of all equipment, copies of all operation manuals, and confirmation that all required utilities such

as electricity, vacuum, and laminar flow hoods were installed properly. Copies of protocols and results for all tests performed by the vendor and on site are maintained in the event of an inspection.

Operational Qualification

The manufacturers of rapid microbiological technologies supply the protocols for the OQ of their instruments. These protocols may be useful as is or may be modified by the end user as appropriate. Typical tests include verifying the interface between the software and the instrument, verifying access to each input message or command processed by the software, cross-checking each external file or data record referenced by supplier, and verifying output message displays, and recorded data generated by the software. These tests were performed and documented with both compendial microorganisms and site-specific environmental microorganisms. Equipment qualification was considered critical at this stage, as it ensured correct operation of the method under working conditions.

Performance Qualification

PQ demonstrated the fitness of the Pallchek for the quantitative (enumeration) and qualitative (growth/no growth after BI sterilizer exposure) tests. Validation experiments were designed to demonstrate and justify the use of the RMMs for testing of BIs. Guidance in PDA Technical Report 33 (PDA 2000) can be followed in performing the validation experiments outlined in Table 3. Testing for each criterion (absence of interference, specificity, limit of detection, ruggedness and repeatability, and robustness) is outlined in protocols with specified acceptance criteria. The performance tests used for the ATP bioluminescence method are summarized below.

Reagent ATP Dilutions

To validate the Pallchek system for ATP detection, serial ten-fold dilutions of reagent ATP were measured. All data were direct liquid measurements on 0.1 mL ATP dilution samples, using two drops of reagent only. Figure 4 summarizes the data. Each dilution included 21 replicates. Readings were taken over a period of four months using different reagent lots. The same operator, instrument, and measurement protocol were used in each dilution series. Total background readings are removed from this data.

Table 3. Experiments for Validation of the Microbial Limit Test Using RMMs.

Experiment	Acceptance Criterion
Absence of Interference (TLV)	The product formulation does not inhibit or enhance method performance, and a TLV has been established.
Specificity	The method has an acceptable ability to detect all the microorganisms with which it is challenged.
Limit of Detection	The method has an acceptable ability to detect microorganisms in samples spiked with 1 to 10 CFU.
Ruggedness and Repeatability	The method demonstrates acceptable ruggedness and repeatability in analyses with different analysts, different instruments, and different reagent batches.
Robustness	The method demonstrates acceptable robustness in analyses with deliberate variations of incubation time, shaking speed, incubation temperature, microbiological physiological conditions, and compositions of mixed cultures.

Accuracy

Pallchek readings are clearly equivalent to dilutions of standard ATP reagents.

Precision and Repeatability

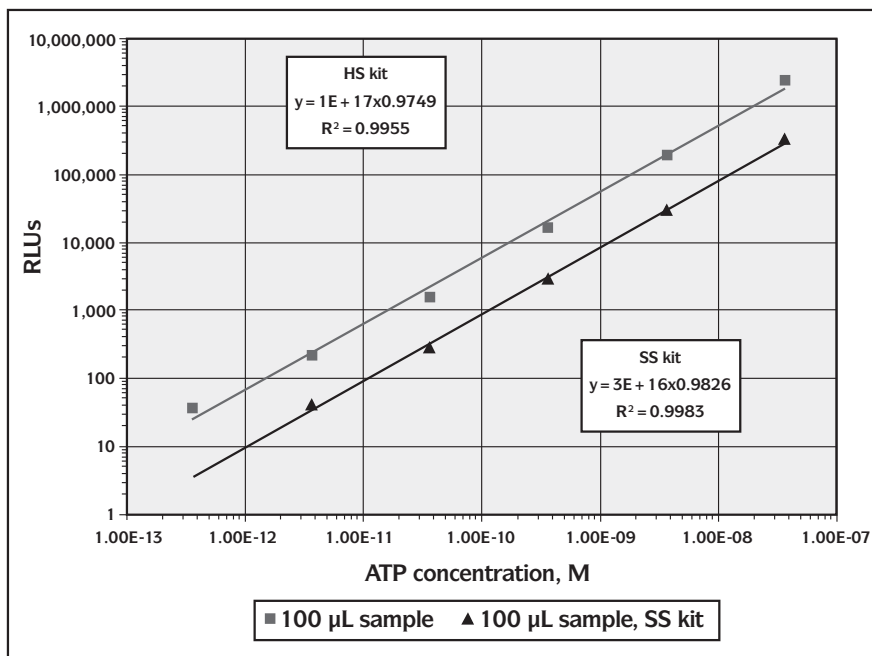
Log standard deviations typically produce low coefficients of variation even over 21 test series. Only at the lowest dilution, which pushes the range of the instrument and represents an extremely dilute sample, does the normal range of the measurements fall outside 70% of the mean.

Precision and repeatability of the instrument and reagent system are clearly excellent.

Limit of Quantification

Correlation to dilution, precision, and repeatability remain acceptable over the six log range of dilutions in the graph. The calculated molar concentration of the most dilute sample is approximately 3.63×10^{-13} M (7.25×10^{-17} mole, this is the average amount of ATP in approximately 40 bacterial cells). At lower concentrations of ATP, RLU readings are not significantly above the range of total background.

Figure 4. Standard ATP Solution in Water (7.25×10^{-7} M) Calibration in Liquid Samples (sample size was 0.1 mL) Using High Sensitivity Kits. Each Data Point Represents an Average of 21 Replicates for All Dilutions.



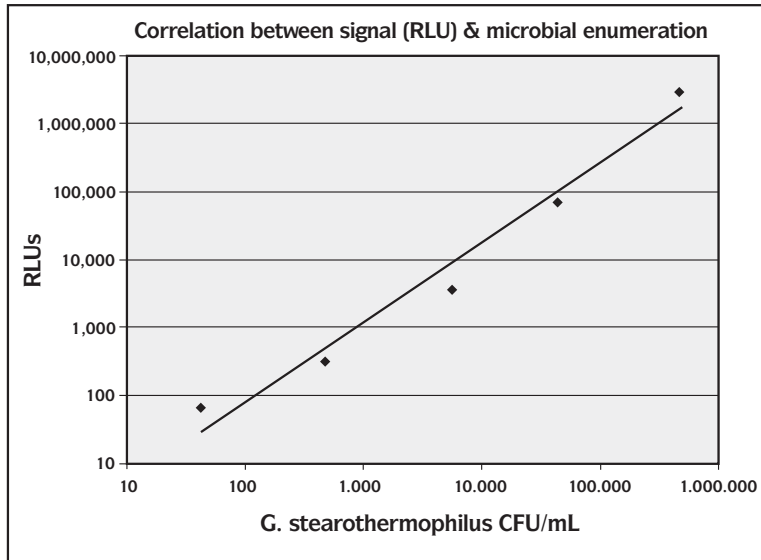
Range and Linearity

The correlation coefficient to dilution is extremely high at 0.9999, and linear over the full six logs of dilution.

Microbial Dilutions – *Geobacillus stearothermophilus*

To validate the Pallchek system for microbial detection, serial ten-fold dilutions of a base culture of *Geobacillus stearothermophilus* were measured. All data were taken on a membrane disk through which each sample had been filtered. Fifteen replicates were included at each dilution. Readings were taken on the same day using the same reagent lot, operator, instrument, and measurement protocol. Total background readings are removed from this data. Figure 5 summarizes the data.

Figure 5: *Geobacillus stearothermophilus* Dilutions in Isotonic Saline (0.1 mL sample) Tested Using High Sensitivity Kits on GN-6 Metricel® Membranes. Each Data Point Represents an Average of 15 Replicates. Water was Used as a Rinse Solution.



Accuracy

Pallchek readings are acceptably equivalent to dilutions of stock *Geobacillus stearothermophilus* culture.

Precision and Repeatability

Log standard deviations typically produce low coefficients of variation even over 15 test series (three different membrane lots). Only at the lowest dilution does the normal range of the measurements fall outside 70% of the mean. Precision and repeatability of the instrument and reagent system are clearly excellent.

Limit of Quantification

Correlation to dilution, precision, and repeatability remain acceptable over the five log range of dilutions in the graph. At greater dilutions of *Geobacillus stearothermophilus*, RLU readings are not significantly above the range of total background.

Range and Linearity

The correlation to dilution is extremely high at 0.9900, and linear over the full five logs of dilution.

Absence of Interference

Interaction of the BI or sample matrix with the luciferase ATP enzymatic reaction (i.e., inhibition or enhancement) was evaluated through tests of non-contaminated samples and samples spiked with the *G. stearothermophilus*. Results from these tests (multiple data points) are used to set the threshold limit value (TLV), above which samples are considered contaminated and below which they are considered not contaminated. The accepted probability of a false-negative result (identifying a contaminated sample as non-contaminated) was 0.00555%. This value is equal to the probability of a false-negative result accepted for the media fill test of sterile drug products.

Specificity

To demonstrate the test's ability to detect microorganisms, at least six replicate samples of sterile biological indicators coupons were spiked with 10 to 100 CFU of the *G. stearothermophilus*. The ability of the test to differentiate between non-contaminated samples and samples containing *G. stearothermophilus* was demonstrated by a two-way analysis of variance, using two levels of the contamination factor (threshold and spore-forming bacteria).

Limit of Detection

The test's ability to detect a range of microorganisms at low concentration was demonstrated using sterile BI coupons spiked with 1 to 10 CFU of *G. stearothermophilus*.

Ruggedness and Repeatability

Precision of the test was demonstrated by analysis of the same sterile BI coupons under a variety of normal test conditions; results were compared for testing with two different analysts, instruments, reagent kit lots, and membrane lots.

Robustness

The ability of the test to yield results unaffected by small but deliberate variations in method parameters was demonstrated by varying the following parameters:

- Incubation time: 0 to 24 hours, using *G. stearothersophilus* BI coupons.
- Shaking speed: 200 or 300 rpm, based on 250 rpm as established by cGMP, using *G. stearothersophilus* BI coupons.
- Incubation temperature: 30°C or 35°C, using *G. stearothersophilus* BI coupons.
- Microbial physiological conditions: soybean-casein digest medium at pH 7.3 or pH 3 and sodium chloride concentration of 0.5% or 5%, *G. stearothersophilus*. The conditions were chosen to challenge the test system, as the bioluminescence enzyme complex is sensitive to both high and low pH and high salt concentration.
- Mixed cultures: (1) Gram-positive and Gram-negative bacteria and a fungus or (2) a spore-forming bacterium and a fungus.

Equivalence

The criterion for equivalence is that the new method be at least as good as the current method. A multi-faceted approach can be used to establish method equivalence, combining review of the published literature, laboratory experiments with spiked samples, and parallel testing with rapid and conventional methods due to the different sensitivity between the rapid method and traditional method.

Test Protocol for BIs Biovalidation and Preliminary Study

A typical test protocol for a BI as pass/fail test for biovalidation studies follows:

- Place a BI strip (*G. stearothersophilus*) into an autoclave and perform the sterilization cycle at 121°C for the established time.
- Aseptically introduce the sterilized strip into 10 mL sterile TSB. Incubate the TSB at 55°C for 24 hours with continuous agitation on an orbital shaker at 250 rpm. Fill the filter funnel with 100 mL sterile WFI and filter. Add the test sample (the 10 mL of TSB containing the strip) and rinse the funnel with 100 mL of WFI. Transfer the membrane into the sample

holder and apply 150 μL of extractant agent to the membrane. Distribute evenly with sterile spreader. Wait approximately 10 seconds.

- Apply 100 μL of the reconstituted bioluminescent reagent to the membrane and distribute evenly using the same sterile spreader.
- Place the Pallchek Luminometer over the membrane and immediately take a reading. Perform the test in triplicate for each sample.
- During the test, it is necessary to perform negative controls using sterile TSB broth and positive controls using low level (<10 spores) of a *G. stearothermophilus* spore suspension using the same analytical protocol.

Table 4 compares the results obtained by Pallchek and the standard method (direct incubation of the BIs strip in TSB). The level of photons generated from the reaction is proportional to the amount of ATP present in the sample analyzed. The photons are measured as RLUs.

Table 4. Comparison Between Pallchek and Traditional Method.

Samples	Time 24 hours RLU/strip	After 14 days CFU/strip
TSB + autoclaved BI strip	1: 2.2×10^2	1: No growth
	2: 1.5×10^2	2: No growth
	3: 2.9×10^2	3: No growth
	average: 2.2×10^2	----
	1: 1.4×10^2	1: No growth
	2: 1.8×10^2	2: No growth
	3: 1.0×10^2	3: No growth
	average: 1.4×10^2	----
Negative control Sterile TSB	1: 1.6×10^2	1: No growth
	2: 1.3×10^2	2: No growth
	3: 1.1×10^2	3: No growth
	average: 1.3×10^2	----

Results obtained by Pallchek confirm the ones obtained using the standard method. A value of 10^2 RLU corresponds to the background level and a non-contaminated sample after incubation for 24 hours at 55°-60°C. These values correspond to absence of growth after 14 days of incubation at 55°-60°C.

Validation of Short Incubation Period BIs Validation

This study was intended to verify a reduction of incubation time for BIs biovalidation using bioluminescence method as the reading system. The testing was intended to reduce the incubation time from the current 14 days to 24 hours incubation. Results from the current studies show that a 24-hour incubation period continues to be accurate. The FDA's guidance requires a 97% compliance (percent of BIs showing growth at intended label claim must be 97% or higher than those BIs showing growth at 14 days). These tests showed 100% compliance.

Procedure

The procedure followed was the FDA guide for Validation of Biological Indicator Incubation Time at www.fda.gov/cdrh/ode/guidance/1320.pdf. A summary of the procedure follows:

1. Determine a "partial kill" cycle in a steam BIER vessel so that after 14 days of incubation, 30% to 80% of the BIs survive.
2. Once the "partial kill" cycle is determined, subject three lots of BIs (steam) from different primary suspensions (batches) to the "partial kill" cycle (with a minimum of 100 units from each lot). For our own verification, we ran more than the required three lots as stated in the protocol. We subjected five lots using recovery media (TSB). Each sample group had 100 units exposed.
3. After exposure, all units were sealed and activated according to user instructions and incubated at 55-60°C for 14 days, with results being checked and recorded every 24 hours.
4. In order to change our incubation time, the protocol states that 97% of all BIs that showed outgrowth in 14 days must also have shown outgrowth by the end of 24 hours.
5. The outgrowth media used was a commercially available Tryptic Soy Broth.
6. The spore species and spore carrier used in this study were the same as those used for our standard products (spore strips).

Results are indicated in Table 5. The full protocol for Reduced Incubation Claim can be obtained at www.fda.gov/cdrh/ode/guidance/1320.pdf.

Table 5. Results from BIs Incubation Time Reduction Validation with Pallchek Compared with Traditional Method.

Lot Number	# BIs Tested	# BIs Positive after 24 Hours using Pallchek	# BIs Positive on Day 14 traditional method
1240	100	71	71
1241	100	64	64
1242	100	86	86
1246	100	77	77
1248	100	75	75

DISCUSSION

For all lots, 100% of those showing outgrowth in 14 days using the traditional method showed outgrowth in 24 hours using bioluminescence method. This exceeds the 97% required by the protocol to sustain a reduced incubation label claim of 24 hours.

CONCLUSION

The study intended to establish a basis for a reduced incubation claim according to the FDA Guidance. Previously, the label claim had been 14 days. The goal was to establish that in "X" amount of time (less than seven days), we would be able to show at least 97% outgrowth of damaged spores with our current media.

The outgrowth evident at 24 hours was 100% of the total outgrowth at 14 days. According to the FDA Guidance, only 97% is required to change the label claim. The above data could be included in the 510(k) submission to make the label claim for BI (steam) a 24-hour incubation period.

Moving from Data to Knowledge

Broad acceptance and implementation of rapid microbiological methods by the pharmaceutical industry will be a slow process. Given the range of skills required, equipment qualification and microbiological performance validation are best

tackled through a team-based approach involving the system manufacturer, to ensure that expertise is effectively handed from the manufacturer to the operators using the equipment in the pharmaceutical sector.

It is essential that during the various stages of evaluation, implementation, and validation, a thorough understanding of the new test system and a true picture of its capabilities be gained. Data generated must be translated into knowledge, which must be effectively documented through a comprehensive validation process and passed on through establishment of a training program.

Future Directions

The current situation for implementation of RMMs is encouraging. New technologies for non-sterile product release testing and pharmaceutical-grade water testing have been approved in both the United States and Europe. The pharmaceutical industry expects that PAT applications for sterile products, continuous environmental monitoring, and biovalidation exercises will be approved in the near future. Continuous, real-time microbial monitoring of environmental conditions, use of barrier technologies, use of robotic technologies, reduction or removal of human intervention steps, and targeted microbial in-process controls will stimulate greater acceptance, and broader applications will create the conditions necessary for replacement of current methodologies, thus moving closer to PAT pharmaceutical manufacturing.

Interest in RMMs in the pharmaceutical industry is high and is expected to increase following reports of successful implementation. Adoption of RMMs is warranted by significant advantages in speed of results, process efficiency savings, sensitivity, and business benefits.

The advent of PAT has given a new impetus to the introduction of RMMs. Conventional microbiological test methods are not capable of delivering real-time or near-real-time results, a prerequisite for successful exploitation of PAT benefits. However, RMMs do have this potential and will be an invaluable aid to successful realization of PAT objectives.

RMM test systems designed specifically to cater to the requirements of the pharmaceutical industry have been developed in close collaboration among suppliers, customers, and regulators and such systems are commercially available. Given FDA's recent approval of this technology as a Microbial Limit Test for drug products and pharmaceutical grade waters, implementation of

these new technologies is now possible. Documents and advice are available to facilitate successful implementation of RMM test systems. Dialogue among suppliers, users, regulatory authorities, and academia is clarifying and defining implementation strategies for such systems, which are being registered and used both in the United States and across Europe.

Conventional methods of microbiological analysis in the pharmaceutical industry have been with us for many years and will continue for some time yet. These methods have been highly successful in determination of microbiological quality attributes for pharmaceutical processes and products. However, the pervading culture of the industry is changing. In the future, successful companies will need to re-examine their attitudes towards process efficiencies, risk taking, and technological innovation. These changes will undoubtedly effect the direction of microbiological analysis. Conventional methods will not be suitable for many future applications. The business drivers that have acted thus far to maintain the status quo are changing:

- Industry attitudes and culture
- Equipment capital costs
- Managerial commitment
- Regulatory environments
- Unclear business benefits

Many of the issues above are being turned on their head due to the emergence of new pharmaceutical manufacturing paradigms. The business environment has moved on and the need for solutions to cut costs and increase competitiveness and efficiencies is irresistible. Pharmaceutical microbiology is part of this process. Obstacles still remain but implementation of RMM systems is beginning to happen. The changes beginning to emerge across the industry represent significant opportunities which will undoubtedly aid the implementation of RMMs both now and in the future.

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He is a speaker at a number of symposiums and conferences in Europe and the US. Mr. Dalmaso is also a Quality System ISO 9000 inspector and an HACCP inspector.