BIOMÉRIEUX

VALIDATION AND SUCCESSFUL IMPLEMENTATION OF SCANRDI®

A Rapid Microbiology Method for Testing the Sterility of Filterable Drug Products

PIONEERING DIAGNOSTICS

Anthony T. Grilli, *President,* FOCUS Laboratories.
177 N. Commerce Way, Bethlehem, PA 18017
(610) 866-7272 AGrilli@FOCUS-lab.com

ABSTRACT

While rapid microbiological methods (RMM) have been in common use by the food, health, and beauty aid industries for decades, the more highly regulated pharmaceutical industry has been slower to adopt them. The FDA supports RMMs for drug testing, as long as these alternative methods are validated to demonstrate their equivalency to the compendial methods. Compounded sterile preparations, with their inherently short shelf lives, are a perfect fit for sterility testing by rapid microbiology methods.

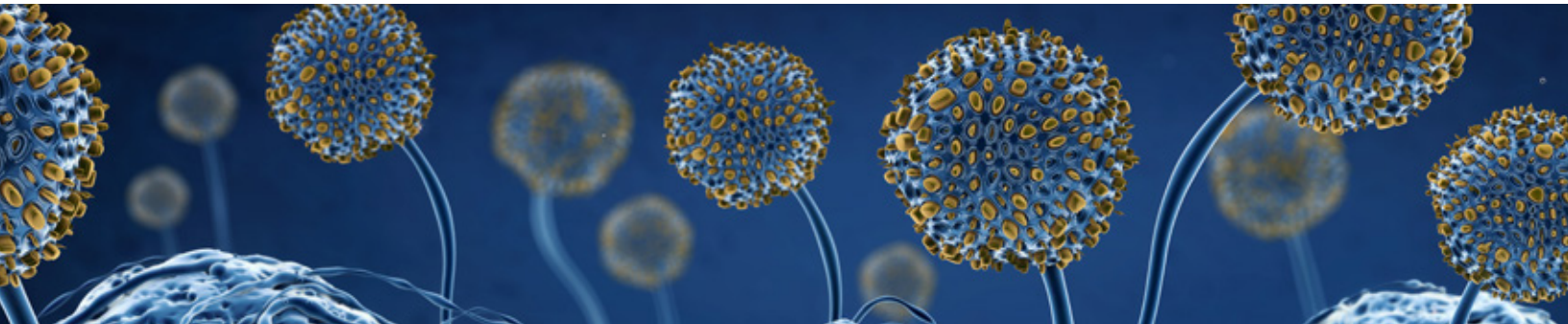
This white paper outlines the process Atlas Analytical used to demonstrate that the SCANRDI® technology is qualified for determining the sterility of test samples.

BACKGROUND

The SCANRDI is a rapid alternative technology for detecting microbial contaminants in filterable drug products. The method utilizes a viability reagent which labels living microbes captured by a filter membrane that does not retain the drug product. The "viability substrate" component of the labeling solution is transported into the microorganism through the cell membrane. Living cells cleave the non-fluorescent viability substrate by an enzymatic esterase reaction, releasing fluorescent molecules (free fluorochrome) within the cell. The cell membrane impermeable to the fluorochrome allows it to be concentrated within the cell and detected by the SCANRDI's laser. Non-living cells are not metabolically active and/or without intact cell membranes are not labeled. These stained microbes are then detected by Laser Scanning Cytometry, and their presence is confirmed by a trained microbiologist using a fluorescence microscope with an automated stage. The whole process is completed within 3-4 hours.

The so-called referee test is the USP <71> Sterility Test, a culture-based test that requires at least 14 days of incubation to confirm sterility. The original culture sterility test was published in the British Pharmacopeia in 1938, and except for media modifications, incubation period extensions, and use of membrane filtration, it remains little changed since. USP <71> is reliant on culturing viable replicating contaminants (aerobic and anaerobic bacteria, and fungi) to a visible endpoint such as turbidity, equivalent to 10^7 colony forming units per mL for most bacteria.

This white paper will outline the qualification and validation process conducted at Atlas Analytical to sterility test and release customers' compounded drug products. Atlas Analytical is a contract testing laboratory which supports sterile compounding pharmacies with cGMP compliant microbiological and analytical testing.



THE PREMISE FOR ALTERNATE MICROBIOLOGY METHODS

While pharmaceutical quality control chemistry tests have evolved over the years from simple wet chemistry titrations and colorimetric reagent spot tests to sophisticated chromatography, inductively coupled plasma and mass spectrometry, the microbiology laboratory down the hall continues to largely use Pasteurian broth and agar methods. This classical approach continues despite encouragement from regulatory agencies to adopt more rapid and accurate methods. The FDA's 2004 Aseptic Processing Guideline, states that "Other suitable microbiological test methods (e.g., rapid test methods) can be considered for environmental monitoring, in-process control testing, and finished product release testing after it is demonstrated that the methods are equivalent to traditional methods."[1]

Similarly, when the FDA introduced the concept of Process Analytical Technology (PAT) to the industry in "Pharmaceuticals for the 21st Century: A Risk based Approach," the agency encouraged innovative approaches for pharmaceutical development, manufacturing and quality assurance. In fact, the first PAT accepted by the FDA was a bioluminescent-based rapid microbiology test. The RMM regulatory acceptance is global, and the draft Revisions to EU Annex 1 states: "The use of rapid microbial methods can also be considered. These methods should be validated for the product(s) or processes concerned and be approved in the registered product testing specification."

The advantage to patient safety of a test that can deliver more accurate results in less than one day versus 14 or more days remains clear. Why has big pharma been slow to develop Rapid Microbiological Methods? The long shelf life of small molecule drug products, up to 3 years, does not encourage more rapid microbiological methods. Compounded drug products do not enjoy the same benefit and prolonged testing consumes the beyond use dating of less than 45 days.

Perhaps the industry hesitation lies in the uncertainty as to how the FDA would interpret validation of a microbiological method. Validation parameters such as limit of detection, specificity and precision have been quite clear to chemists for years, but become less clear in microbiology where the scientists are more comfortable with colony forming units and most probable numbers. If USP <71> Sterility Test is the referee method, how does one go about comparing it to an alternative method? A result of no growth in a compendial method does not mean in absolute terms that no cells are actually present in the culture. The actual limits of detection cannot be established quantitatively, as microbiology is a logarithmic science, only precise to 0.3 – 0.5 $\log_{10}$. And it is understood that many variables affect the reliability of conventional methods, including selection of growth media, incubation conditions, nutritional requirements of microorganisms, and the physical condition of microorganisms.

The USP answered this need for clarification with its general Informational Chapter <1223> Validation of Alternative Microbiological Methods. For microbiological test validation, they outlined the following:

Validation Parameter	Qualitative Test	Quantitative Test
Accuracy	No	Yes
Precision	No	Yes
Specificity	Yes	No
Limit of Detection	Yes	Yes
Limit of Qualification	No	Yes
Linearity	No	Yes
Range	No	Yes
Repeatability	Yes	Yes
Ruggedness	Yes	Yes

[1] FDA Aseptic Guidance

The USP <71> Sterility Test is qualitative, therefore Atlas Analytical used the qualitative test criteria outlined by USP <1223> to validate the SCANRDI®.

The manufacturer of the SCANRDI, bioMéreiux, filed a Drug Master File (DMF) with the FDA for the SCANRDI System. They provided comprehensive validation data in the DMF, including specificity, limit of detection, repeatability and ruggedness (Termed Performance Qualification 1) that are difficult for a sterile compounding pharmacy to generate. Validation strategy for compounding pharmacy is to reference bioMérieux's data from the DMF but demonstrate method suitability for every specific drug product and formulation they compound (Termed Performance Qualification 2).

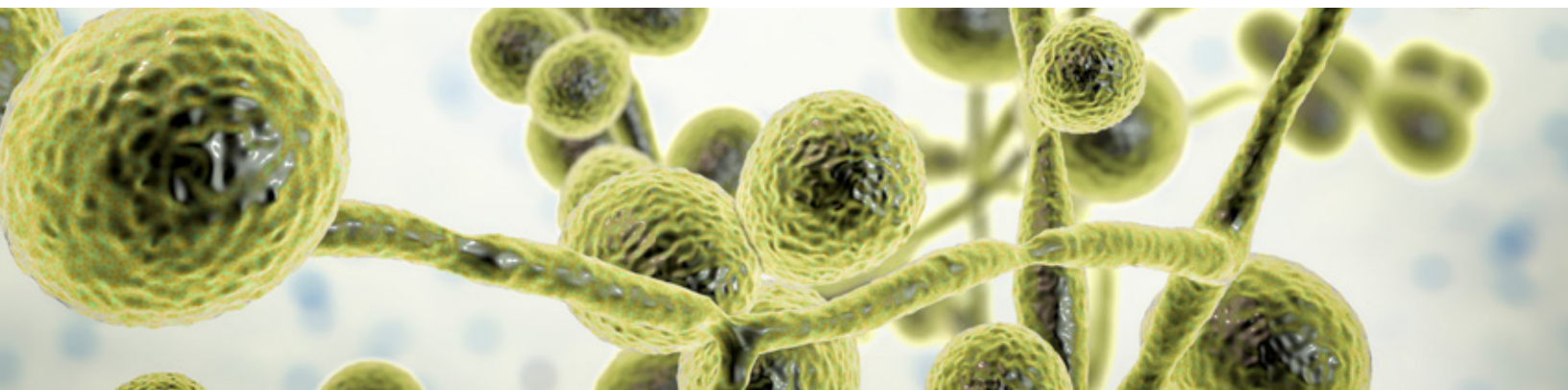
Performance Qualification 1 (bioMérieux Responsibility)	Performance Qualification 2 (Testing Laboratory Responsibility)
Limit of Detection	Analyst Qualification
Specificity	Product Suitability
Robustness/Ruggedness	Method Suitability

LIMIT OF DETECTION

Diluting chemical analytes to discrete amounts, such as 1 ppm or 1 ppb, is routine when validating an analytical instrument. Not so for microbial methods, where colony forming units and microbial dispersion prove this specific dilution more problematic. bioMérieux resolved this challenge by diluting the USP <71> challenge organisms to extinction and testing both methods' ability to detect their frequency of presence/absence. Each organism was tested 18 times. Each inoculum was verified to have a concentration of <5 cfu when analyzed by the plate count method. A chi-square test was performed to confirm the frequency of detection with the SCANRDI system was comparable to the USP <71> sterility test.

SPECIFICITY

The USP defines specificity as the ability to detect a range of microorganisms, demonstrating that the method is fit for its intended use. The basis of the SCANRDI technology is that living microbes hydrolyze a fluorogenic substate liberating a fluorochrome that fluoresces when activated by laser scan. By its nature, it is specific to all viable microbes. bioMérieux demonstrated this specificity by detecting a wide range of bacteria, yeast, mold, anaerobes and spores. Atlas replicated the specificity of this technology by detecting a range of QC organisms and environmental isolates in its own lab, including *Pseudomonas aeruginosa, Escherichia coli, Salmonella enteritida, Candida albicans, Aspergillus brasiliensis, Clostridium sporogenes, Saccharomyces cervesiae, Bacillus subtilis, Penicillium roquefortii, Micrococcus lutea* and *Micrococcus luteola.*



ROBUSTNESS/RUGGEDNESS

bioMérieux proved through laboratory testing that small variations in method parameters, instruments, batches of reagents and laboratories had no impact on the performance of the SCANRDI® system. Ruggedness was shown by using three different systems, three different operators and two different batches of reagents and consumables.

ANALYST QUALIFICATION

In the end, any method is only valid if the human operator interacting with it is properly trained and qualified. Atlas Analytical performs all the necessary requisite qualifications prior to an analyst entering a clean room to perform Sterility Testing: gowning qualification, environmental monitoring, aseptic transfer verification. But Atlas also confirms an analyst's ability to accurately deliver <100 cfu of test species to a SCANRDI filtrate, and Atlas confirms an analyst's ability to discriminate between fluorescent particles and viable microorganisms.

PRODUCT SUITABILITY

Atlas Analytical confirms that each compounded formulation it tests is suitable to be tested by SCANRDI. Suitability testing confirms that the product is filterable, does not autofluouresce, and does not contain >50 nonspecific fluorescent particles per filter.

METHOD SUITABILITY

Atlas Analytical confirms that SCANRDI will be able to detect each of the six USP <71> test species in the presence of sample filtrate. The organisms are introduced to the USP Fluid D wash after sample filtration, in the same fashion that it is introduced in the USP <71> Microbial Suitability Test. This validation is usually performed on three different lots of product.

AUTHOR BIOGRAPHY

Anthony T. Grill holds a Master of Science degree in Microbiology from Rutgers University. He has more than 30 years of industrial microbiology experience and has managed contract laboratory operations for Celsis Laboratory Group and SGS. He has worked with and validated bioluminescent, immunological, and chemiluminescent rapid microbiological methods. He is owner of FOCUS Laboratories and Atlas Analytical Inc., two laboratories dedicated to excellent microbiology testing support to all segments of the Life Science Industry. He is currently serving as President of the Metro Chapter of PDA.

Smith R, Von Tress M, Tubb C, Vanhaecke E. Evaluation of the ScanRDI as a Rapid Alternative to the Pharmacopoeial Sterility Test Method: Comparison of the Limits of Detection. PDA J Pharm Sci Technol. 2010;64(4):356-363.

bioMérieux, Inc. • 100 Rodolphe Street • Durham, NC 27712 • U.S.A. • (800) 682-2666
www.biomerieux-usa.com