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Microbiology Series – Part 1

NEW YEAR, OLD CHALLENGES!

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This is the first of many articles in our continuing series on Rapid Microbiological Methods that will appear in *European Pharmaceutical Review* during 2012. For the past two years, I have enjoyed sharing with you a broad range of topics associated with the validation and implementation of rapid microbiological methods (RMMs), including:

- A review of the history of conventional microbiology and the benefits of using RMMs
- Validation strategies
- Perspectives from the regulatory authorities, including FDA and EMA
- Overviews of currently available technologies, including those based on the growth of microorganisms, detection of cellular targets, optical spectroscopy, nucleic acid amplification and gene sequencing, viability staining and laser excitation, as well as micro-electro-mechanical systems, or MEMS

In addition to my articles, numerous companies have published their success stories of RMM selection, validation and implementation, for a variety of applications including, but not limited to, sterility testing, bioburden analyses, water testing, environmental monitoring and the detection of Mycoplasma and other microorganisms. With all of this available information and practical guidance in our hands, it stands to reason that we should now expect a massive undertaking by the industry to put these novel technologies in their rightful place. At least that's what this author wants to believe. Unfortunately, the acceptance of rapid methods continues to be hampered by misconceptions, misunderstandings, preconceptions, biases, prejudices, prejudgments, and above all, myths. The harsh reality is that many in our industry would rather promote doubt over acknowledgment that rapid methods may actually lead to a better quality product, manufacturing efficiencies, significant returns on investment and increased process knowledge. And in an era where product quality is at the forefront of patient safety, rapid methods should be considered as analytical tools to monitor and, under the right strategy, control adventitious contamination. Propagating the so-called 'myths' associated with RMM implementation should once and for all be terminated, and this is the focus for my first article of the New Year.

Myths and rapid methods

A myth can take on many forms including a widely held but false belief or idea, a misrepresentation of the truth, a fictitious or imaginary person or thing, or an exaggerated or idealised conception of a person or thing. Many myths associated with rapid methods can fall under each of these categories. For example, widely held but false beliefs or ideas include:

- Rapid methods are not accepted by regulatory authorities
- They do not support QbD or PAT principles or initiatives
- They will never replace pharmacopoeial tests
- There is little validation guidance
- RMMs offer no return on investment

Similarly, rapid method myths that foster a misrepresentation of the truth include:

- Data from RMMs will exceed our specifications and action levels, which will translate to an increase in batch rejections
- Changing acceptance levels will not be allowed
- It will take forever to gain regulatory approvals
- There's just not enough information or guidance out there

Some in the industry do not want to explore the use of a rapid method unless it detects, enumerates and identifies viable microorganisms at the single cell level while doing this all at the same time. This sounds like a fictitious or imaginary thing to me. Finally, the notion that rapid methods will solve all of your contamination and product quality issues is an exaggerated or idealised conception. Yes, RMMs can provide a better understanding of your contamination control efforts and offer enhanced process and product knowledge (from a microbiological

perspective), but RMMs are analytical tools to help us get there, and not the end all.

The impact on industry

Although companies have successfully validated and implemented rapid methods, both in the US and throughout Europe, many firms don't want to be the first on their block to go down this path. Or, they believe it will cost too much and that the regulatory authorities won't understand the concepts. Additionally, we've been doing just fine all this time, right (i.e., if it 'ain't' broke, why fix it?)? Furthermore, we proclaim to embrace 21st Century manufacturing technologies to deliver 21st Century quality product, but continue to use 19th Century microbiology methods. If you were external to our industry, would you trust us to make quality pharmaceuticals, given the fact that we continue to address contamination problems which impact our production lines and in some cases, our products? It's time to move forward and accept the next generation in microbiology testing. But first, we have to debunk these rapid micro method myths.

Myth: RMMs are not accepted by the FDA

Regulatory acceptance is the industry's greatest fear and bewilderment. The authorities not only understand, but also embrace and encourage the use of RMMs. Whether it be the FDA, EMA, Japanese PMDA or Australian TGA, policy and guidance have been modified or introduced that promote the use of alternative microbiological methods, and even make it easier to get them approved. For example, the FDA's *Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice*, recommends the use of rapid genotypic methods for microbial identification because they have been shown to be more accurate and precise than biochemical and phenotypic techniques. The guidance also states that other suitable microbiological tests (e.g., rapid methods) can be considered for environmental monitoring, in-process control and finished product release testing, as long as they have been demonstrated to be equivalent or better than the conventional methods.

In 2008, FDA's Center for Biologics came out with a draft guidance entitled the *Validation of Growth-Based Rapid Microbiological Methods for Sterility Testing of Cellular and Gene Therapy Products*. The document provides a roadmap for demonstrating that an alternative, growth-based RMM is equivalent to the conventional sterility test method.

More recently, the US Federal Register proposed to amend the sterility test requirements for biological products because *"Advances in technology in recent years have allowed the development of new sterility test methods that yield accurate and reliable test results in less time and with less operator intervention than the currently prescribed culture-based methods."* Furthermore, this 2011 proposal is supported by internal assessments of rapid methods within FDA's own test laboratories.

And most recently, the FDA published their new strategic plan for regulatory science, which includes the development of *"sensitive, rapid, high-throughput methods to detect, identify, and enumerate microbial contaminants and validate their utility in assessing product sterility."*

Clearly, the FDA accepts the use of rapid methods. And the Agency has encouraged the industry to openly discuss their validation and implementation strategies with FDA microbiologists, in addition to using filing and notification tools such as comparability protocols and a reduced reported structure for communicating when the RMM is being implemented (e.g., Changes Being Effected-0, or CBE-0).

Myth: RMMs are not accepted by European regulators

The European regulators have expressed their

acceptance of RMMs for a number of years. At a PDA RMM meeting in 2009, Riccardo Luigetti (EMA) stated that RMMs clearly have the potential to be used to support QbD, and that the introduction of these methods is generally supported by the EU regulatory competent authorities. Additionally, Paul Hargreaves (MHRA) has communicated that his agency has actively encouraged the pharmaceutical industry to implement RMMs in order to improve patent safety.

In 2005, the EMA Quality Working Party provided guidance on the use of alternative methods for the rapid control of microbiological quality of WFI and purified water. Since it is expected that the water will continue to meet Ph. Eur. specifications, if tested, the QWP stated that no change to dossier requirements should be required (depending on the level of detail in the original dossiers concerned) and that no regulatory impact on individual products would be anticipated.

A more recent change to EMA policy includes the introduction of the Post Approval Change Management Protocol (PACMP). This is similar to FDA's comparability protocol, where the proposed RMM validation test protocol is submitted to EMA for approval (as a Type II Variation), and then the subsequent data is submitted as a Type IA or IB variation. This new protocol approval process takes much of the uncertainty out of the equation with regard to whether a validation strategy and test plan is acceptable, which was a deterrent for many companies in the past (previously, companies would submit their validation data and hope that they would not have to repeat the test or significantly change the test strategy following EMA review). Moreover, under the new PACMP procedure, data submitted as a Type IA variation can allow the RMM to be implemented immediately, which is similar to FDA's CBE-0 notification process.

Myth: RMMs have no impact on product quality

For many years, our industry has successfully provided pharmaceuticals to the public using batch processing with laboratory testing conducted on collected samples to ensure product quality. However, we are not an industry without contamination events, and some firms have faced significant microbiological control issues, which have resulted in facility closures and even product recalls. So where would RMMs fit in? Quality by Design (QbD) and Process Analytical Technology (PAT) principles teach us that we can use analytical tools to implement 'real time' quality control, increase our level of automation and ensure predefined product quality at the end of the manufacturing process by monitoring and controlling the process as it happens. Rapid methods can be used for real-time or close to real-time microbial detection in environmental monitoring, process water, fermentation and a variety of in-process bioburden samples. That's the monitoring piece in PAT. The control piece occurs when we observe a contamination event, as it is transpires, because RMMs allow us to respond much faster than if we were using conventional methods (actually, we wouldn't be able to respond in a timely manner when using conventional methods because our results wouldn't be available for days after the event occurred!). Now we can stop the manufacturing process, investigate the issue in real-time, be in a better position to quickly resolve the issue, segregate the affected product or in-process material (when appropriate), and once we are back in control, continue with production.

Furthermore, the data generated from many RMM technologies are rich with information, and can subsequently be used to enhance our process knowledge. Now we can be proactive in continuously improving our processes and engineer potential contamination out of the picture.

Myth: RMMs will never replace finish product testing

It already has. A number of companies have obtained regulatory approval (FDA and EMA) for using rapid methods as an alternative to the compendial sterility test. And other firms have used rapid method technologies for the release of non-sterile pharmaceutical preparations, instead of waiting for the results of a compendial Microbial Limits Test. This is only the beginning.

Myth: there's not enough validation guidance

There are currently three guidance documents for the validation of rapid methods: PDA Technical Report #33, USP <1223> and Ph. Eur. 5.1.6. For a number of years, firms have utilised these documents to successfully validate, gain regulatory approval and implement their RMMs. I will note that each of these documents is currently under revision, but the overall guidance should not change significantly.

Myth: there's no return on investment

There has always been the perception that the cost of RMM capital equipment and validation will far outweigh the cost savings. However, no one, not even your production manager, site head or COO, can make that assumption unless you have performed a financial analysis, comparing the overall costs associated with the existing method and the proposed RMM. Truth be told, some companies have realised huge savings when implementing rapid methods. This will be a topic I will cover in great detail later this year.

Myth: we can't change our acceptance levels

Some RMMs, especially those that do not rely on microbial growth, may provide a higher recovery count as compared with traditional methods. Furthermore, some RMM measurements may be completely different from what we have been historically used to (e.g., fluorescent units vs. CFU). For example, Dr. David Hussong (FDA) has stated that rapid methods may rely on a completely different body of information; some may be direct measurements, some indirect. In either event, previous acceptance criteria may not be applicable. Therefore, implementation of newly developed, or more rapid, microbiology methods may also require establishment of new acceptance criteria. Handling acceptance levels is something you can include in your validation and implementation strategy, and if you have any doubt, I encourage you to discuss this topic with the relevant regulatory authorities.

Myth: we need the Holy Grail

I have heard, on more than one occasion, that a company will only consider a RMM if it detects, enumerates and identifies viable micro-organisms at the single cell level and do each of these tasks all at the same time. Wake up people. We are getting close, but the state of the art is not quite there yet. For example, Raman spectroscopy is in a good position to be able to detect, count and ID single cells that are captured on a membrane. But more work needs to be done, and RMM technologies will catch up with our desires. In the meantime, many companies have employed one RMM for enumeration, and a separate RMM for identification or the detection of specific organisms of interest. And there are acceptable strategies for using 'destructive' RMMs, or RMMs that do not capture microorganisms, for subsequent testing. Simply put, unless you are

Robert Langdon, Indiana Jones or Monty Python, leave the Holy Grail for another day.

Myth: RMMs will solve all of your contamination problems

No, not all of them, but RMMs may be able to help you understand why you are having contamination events, what the root cause is, and how to resolve the issues. Rapid methods can support a comprehensive contamination control program, and when contamination arises, they can be used as investigative tools. I do believe that if RMMs were more widely used within the industry, a number of firms may have had a better opportunity to detect contamination issues, remedy the situation and prevent future contamination from occurring, long before those same companies were forced to shut down manufacturing lines, close their facilities, or recall their products because of the presence of microorganisms.

Myth: there's not enough information about RMMs

I already mentioned the availability of three guidance documents for RMM validation, but there are other sources of information in the public domain. Publications, such as the one you are reading, have provided papers on RMM validation, implementation, technologies and other topics of interest. There are conferences dedicated to rapid methods, as well as online user groups (e.g., 'Rapid Micro Methods' on *LinkedIn*). Another comprehensive resource for all things rapid methods is my own educational website, http://rapidmicromethods.com. Here you will find a holistic overview of validation strategies, regulatory expectations, return on investment guidance, scientific tutorials, current RMM news, a calendar of events, newsletter and my blog. A recent addition is the RMM Product Matrix, where you can compare more than 50 different rapid method technologies, with details including scientific methods, applications, time to result, throughput, sensitivity, organisms detected, identification libraries and product workflow, arranged in three separate comparison tables (microbial identification, qualitative and quantitative methods).

Summary

After reading this article, I hope you agree that most rapid method myths that have been circulating throughout the industry are just not true. Regulatory authorities want RMMs implemented, and their use is directly aligned with the future state of pharmaceutical manufacturing, QbD, PAT and continuous process and product improvement. There is validation guidance and this guidance will become clearer. Also, the cost of implementation can be a good investment. So stop listing to the naysayers, cynics, sceptics and worrywarts, and embrace microbiology for the 21st Century. I have a feeling our friend Louis Pasteur would agree!`

BIOGRAPHY

Dr. Michael J. Miller is currently the President of Microbiology Consultants, LLC (http://microbiologyconsultants.com). For more than 23 years, he has held numerous R&D, manufacturing, quality, and consulting and business development leadership roles at Johnson & Johnson, Eli Lilly and Company, Bausch & Lomb, and Pharmaceutical Systems, Inc. Dr. Miller consults with multinational companies in providing technical, quality and regulatory solutions in support of RMMs, sterile and non-sterile pharmaceutical manufacturing, contamination control, isolator technology, validation and microbiological PAT. He also provides comprehensive training for his clients in the areas of rapid method validation and implementation. Dr. Miller has authored over 100 technical publications and presentations, is the editor of PDA's *Encyclopaedia of Rapid Microbiological Methods*, and is the owner of http://rapidmicromethods.com. He currently serves on a number of PDA's program and publication committees and advisory boards, is co-chairing the revision of PDA Technical Report #33: *Evaluation, Validation and Implementation of New Microbiological Testing Methods*. Dr. Miller holds a PhD in Microbiology and Biochemistry from Georgia State University (GSU), a BA in Anthropology and Sociology from Hobart College, and is currently an adjunct professor at GSU. He was awarded PDA's Distinguished Service Award and was named Microbiologist of the Year by the Institute of Validation Technology (IVT).

RAPID MICRO METHODS AND EMA'S POST APPROVAL CHANGE MANAGEMENT PROTOCOL

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This is the second paper in our continuing series on Rapid Microbiological Methods that will appear in *European Pharmaceutical Review* during 2012. In my last article, we discussed a number of myths or misconceptions associated with the validation and implementation of rapid microbiological methods (RMMs). In fact, most RMM myths that have been circulating throughout our industry are not true or have been exaggerated to the point that many companies continue to be hesitant in exploring what RMMs have to offer.

One of the most prominent myths is that the regulators do not understand, accept or even encourage the use of rapid methods. I submit to you that the regulators want to see RMMs implemented, as their use is directly aligned with the future state of pharmaceutical manufacturing, QbD, PAT and continuous process and product improvement. Furthermore, recent changes to regulatory guidance and proposed policy have made it easier to implement RMMs than ever before. In my last article, I introduced a relatively new process that the European Medicines Agency (EMA) launched that allows for the review and approval of RMM validation strategies before testing is initiated. A more thorough review of this process, better known as the Post Approval Change Management Protocol (PACMP), is presented herein.

History

One reason for an apparent lack of willingness to move microbiology technology forward has been the industry's perception that the European regulatory framework actually hinders, instead of encourages, the imple-

mentation of RMMs. For example, many end-users consider the European regulatory environment for submissions as being more complicated than and not as straightforward as the procedures they have used for RMM approvals in the US.

Although individual member states have approved RMMs for routine use, many of the tools provided by the FDA have not existed within the EMA. Additionally, there was no equivalent to the FDA Comparability Protocol in Europe, and companies have had no formal process for submitting a RMM validation strategy for review and approval prior to the initiation of the actual testing plan. For some firms, it has historically taken between 12 – 18 months for their RMM validation dossier to be reviewed and commented on by numerous member states, thereby extending the time (and cost) to revise validation plans, repeat testing, and implement the RMM. Fortunately, significant regulatory policy changes have recently been introduced which now pave the way for a friendlier and simpler strategy for RMM validation and implementation within Europe, and this strategy is very similar to the use of FDA's Comparability Protocol. But before we review this new policy, it is appropriate to understand what a Comparability Protocol is and how firms have used this tool to obtain validation plan reviews and approvals.

> "Although individual member states have approved RMMs for routine use, many of the tools provided by the FDA have not existed within the EMA"

FDA Comparability Protocol

A Comparability Protocol (CP) is a well-defined, detailed, written plan (and prior-approval supplement) for assessing the effect of specific CMC (Chemistry, Manufacturing, and Controls) changes in the identity, strength, quality, purity and potency of a specific drug product as these factors relate to the safety and effectiveness of the product. The CP describes the changes that are covered under the protocol and specifies the tests and studies that will be performed, including the analytical procedures that will be used, and acceptance criteria that will be achieved to demonstrate that specified CMC changes do not adversely affect the product. In terms of RMMs, the CP is a validation protocol to demonstrate that the RMM is suitable for its intended use (i.e., as an alternative to the current microbiological testing method used with the drug product). Furthermore, the CP can be particularly useful for changes of a repetitive nature, such as the use of an RMM for multiple products or processes.

Because the FDA reviews the CP, deficiencies in the validation plan can be corrected prior to performing the studies, eliminating the need to repeat some or all of the testing. Once the FDA approves the CP, the experiments are carried out, and if they meet the acceptance criteria provided in the CP, the FDA is notified via a Special Report [as per 21 CFR 314.81(b)(3)(ii)], the latter submitted to t he relevant application(s). The Special Report references the approved CP and includes a brief description of the RMM and its use, confirmation that the acceptance criteria have been met, and the date of implementation. The report can be as small as one page, because there is no need to communicate any of the testing data back to the FDA. Additionally, a reduced reporting category can be used to notify the FDA that the RMM is being implemented, such as a Changes Being Effected (CBE)-30 or CBE-0. For example, when using a CBE-0 notification process, a firm can immediately implement the RMM for routine use.

Finally, the same CP can be used (without going through another review and approval process) to subsequently validate the same RMM for additional products or samples, as long as the CP acceptance criteria are met. In this case, the same approved reduced reporting notification method can be used. It should also be noted that a number of companies have already used these same strategies for RMM approvals associated with drug product that is sold in the US.

EMA's answer to the Comparability Protocol

As previously discussed, the EMA did not have a policy in place that allowed for the review and approval of a RMM validation plan prior to conducting the actual validation studies. Historically, the evaluation of a proposed variation was performed as a whole, meaning that the planned studies, methods and acceptance criteria were simultaneously submitted with the testing results. If questions arose by any of the competent authorities, the submission may have been delayed due to additional testing requirements. However, in early 2011, the EMA introduced significant changes to the management of RMM reviews that should make the validation and approval process much more predictable and in-line with the processes currently used by the FDA. The new process, which is very similar to FDA's Comparability Protocol, is called the Post Approval Change Management Protocol (PACMP).

In this new, two-step process, a change management testing protocol is first submitted as a Type 2 Variation. Commission Regulation (EC) No. 1234/2008 ('the Variations Regulation') and the 'Commission guideline on the details of the various categories of variations' ('the Classification Guideline') defines a Type II variation as a 'major variation', which may have a significant impact on the Quality, Safety or Efficacy of the medicinal product. The protocol should include the overall testing strategy, such as the planned studies, acceptance criteria and methods. Prior to submitting the PACMP, a firm may also discuss their testing strategies with the EMA under the Scientific Advice procedure. Once the protocol is approved, the submitting company will perform the testing as specified in the protocol.

> "Significant regulatory policy changes have recently been introduced which now pave the way for a friendlier and simpler strategy for RMM validation and implementation within Europe"

The second step of the PACMP process involves submitting the resulting data (assuming they have met the protocol's acceptance criteria) as either a Type 1A or 1B Variation. The decision as to whether the data is submitted as a Type 1A versus a Type 1B variation is determined at the time of protocol review and approval.

Type IA variations are considered as minor variations that have only a minimal impact, or no impact at all, on the quality, safety or efficacy of the medicinal product, and do not require prior approval before implementation. If the data is submitted under a Type 1A variation, the company can immediately implement the rapid method, similar to what the FDA would consider under a CBE-0. The EMA also refers

to this implementation strategy as the 'Tell and Do' procedure.

Type IB variations are also considered as minor variations, but is neither a Type IA variation nor a Type II major variation. In fact, when one or more of the conditions established in the Annex to the Classification Guideline for a minor variation of Type IA are not met, the concerned change may be submitted as a Type IB variation unless the change is specifically classified as a major variation of Type II. Specific supporting data for Type IB variations will depend on the specific nature of the change.

> "In terms of RMMs, the CP is a validation protocol to demonstrate that the RMM is suitable for its intended use"

Type IB variations must be notified to the National Competent Authority / EMA by the Marketing Authorisation Holder (MAH) before implementation, but do not require a formal approval. Furthermore, the MAH must wait a period of 30 days to ensure that the notification is deemed acceptable before implementing the change. The strategy is considered as a 'Tell, Wait and Do' procedure, and is similar to FDA's CBE-30.

The most apparent difference between the EMA's PACMP and FDA's Comparability Protocol is that the former requires the submission of the test data. In any case, this is still a much more desirable RMM validation process than what companies were required to follow in the past.

Summary

The implementation of RMMs represents significant progress toward the acceptance of microbiological PAT and QbD solutions for the industry, and is directly aligned with the expectations for pharmaceutical manufacturing, quality and operational excellence in the 21st Century. Whether a firm plans on satisfying the expectations of the FDA, EMA or any other regulatory agency, it is still important to discuss your RMM qualification and implementation plans early in the design phase to ensure that the best strategy is agreed upon. You may even find that the use of a PACMP or Comparability Protocol may not even be required, depending on the rapid method technology, its application(s) and/or the products or materials on which the RMM will be used. This may be most applicable for test samples and their specifications that are not included in a regulatory dossier, such as in-process sample matrices.

BIOGRAPHY

Dr. Michael J. Miller is currently the President of Microbiology Consultants, LLC (http://microbiologyconsultants.com). For more than 23 years, he has held numerous R&D, manufacturing, quality, and consulting and business development leadership roles at Johnson & Johnson, Eli Lilly and Company, Bausch & Lomb, and Pharmaceutical Systems, Inc. Dr. Miller consults with multinational companies in providing technical, quality and regulatory solutions in support of RMMs, sterile and non-sterile pharmaceutical manufacturing, contamination control, isolator technology, validation and microbiological PAT. He also provides comprehensive training for his clients in the areas of rapid method validation and implementation. Dr. Miller has authored over 100 technical publications and presentations, is the editor of PDA's *Encyclopaedia of Rapid Microbiological Methods*, and is the owner of http://rapidmicromethods.com. He currently serves on a number of PDA's program and publication committees and advisory boards, is co-chairing the revision of PDA Technical Report #33: *Evaluation, Validation and Implementation of New Microbiological Testing Methods*. Dr. Miller holds a PhD in Microbiology and Biochemistry from Georgia State University (GSU), a BA in Anthropology and Sociology from Hobart College, and is currently an adjunct professor at GSU. He was awarded PDA's Distinguished Service Award and was named Microbiologist of the Year by the Institute of Validation Technology (IVT).

RAPID STERILITY TESTING AND THE IMPACT OF RECENT CHANGES TO THE US CODE OF FEDERAL REGULATIONS

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This is the third paper in our continuing series on Rapid Microbiological Methods (RMM) that will appear in *European Pharmaceutical Review* during 2012. Rapid sterility testing is one of a number of applications that novel microbiological technologies afford the pharmaceutical industry. RMM technologies have already been validated and implemented for both small and large molecule pharmaceuticals and ophthalmic products, in addition to cell therapy and tissue culture products, as an alternative to pharmacopeial sterility tests, and company success stories have been presented and published at numerous professional meetings and in a variety of scientific journals (please see the reference page at http://rapidmicromethods.com for the full titles). However, the industry as a whole has not embraced the use of rapid sterility testing as much as other microbiological applications, such as in-process bioburden, environmental monitoring and Microbial Limits testing. The reasons are varied, and have included concerns regarding return on investment, the extent of the validation plan and regulatory acceptance. Fortunately, recent changes in regulatory policy make it clear that RMMs for finished product sterility testing have a place in our industry, and it is the FDA that is leading the motivation for change.

In February 2008, the FDA published their draft guidance on the validation of growth-based RMMs for sterility testing of cellular and gene therapy products. The guidance addressed considerations for method validation and determining equivalence of an RMM to sterility assays described in Title 21 Code of Federal Regulations (CFR), 610.12 (21 CFR 610.12). Additionally, the guidance specifically applied to somatic cellular therapy and gene therapy products that are regulated within the Center for Biologics Evaluation and Research (CBER) or other products that are also subject to sterility testing under 21 CFR 610.12. However, the guidance was not intended for human cells, tissues, and cellular and tissue products (HCT/Ps), HCT/Ps which are regulated as medical devices under 21 CFR Part 820, or for other pharmaceutical products that would normally be regulated by the Center for Drug Evaluation and Research (CDER).

The FDA realised that many cell-based products couldn't be cryopreserved or otherwise stored without affecting viability and potency. Additionally, most cell-based products are manufactured using aseptic manipulations because they cannot undergo sterile filtration or terminal sterilisation. Furthermore, rapid and effective testing was needed because many cell-based products have a potentially short dating period, which often necessitates administration of the final product to a patient before sterility

test results are available. Because of the challenges associated with cell-based products, there was a significant need to develop, validate and implement sterility test methods that are more rapid than the sterility test methods described in 21 CFR 610.12.

For these reasons, the draft guidance provided direction on how to demonstrate that an alternative or rapid method is equivalent to a test method specified in 21 CFR Part 610, such as the sterility testing described in 21 CFR 610.12. It was also expected that an applicant demonstrate in a Biologics License Application (BLA) or supplement to a BLA that the alternative method will provide assurances of the safety, purity, potency and effectiveness of the biological product equal to or greater than the assurances provided by the specified method (21 CFR 610.9).

It is also important to note that the principles of RMM validation described in the draft guidance applied only to growth-based RMMs. Growth-based RMMs, like traditional methods of detecting viable microorganisms as described in 21 CFR 610.12, rely on the ability to recover and detect organisms from the product and demonstrate their viability by multiplication in liquid media. Therefore, the specific recommendations in this draft guidance document may not have been applicable for non-growth-based RMMs that detect microbiological surrogates, such as Adenosine Triphosphate (ATP) or nucleic acids. For these reasons, the guidance focused solely on RMMs that provided qualitative results (i.e., detection of microorganisms).

The guidance also stated that RMMs have the potential to replace the traditional methods for microbiological testing in the manufacturing process, including component (e.g., raw material, excipient) testing, in-process testing, drug substance testing and drug product in its final container.

Reliance on validated sterility testing methods is a critical element in assuring the safety of a product. Therefore, the draft guidance specified that proper validation of critical methods, including RMMs, demonstrate that the methods are suitable for their intended purpose and provides assurance that the results obtained are accurate and reproducible. Also included in the guidance was an overview of validation criteria that should be assessed, including limit of detection, specificity, ruggedness and robustness, what micro-organisms to use, controls, and what method comparison studies to consider.

Proposed changes to the US Federal Register

In June 2011, CBER continued to progress their proposed usage of RMMs from the 2008 draft guidance and into the US CFR. In this proposal, the FDA recommended amending the sterility test requirements to provide manufacturers of biological products greater flexibility and to encourage use of the most appropriate and state-of-the-art test methods for assuring the safety of biological products. They took this action as part of FDA's continuing effort to review and, as necessary, update the biologics regulations.

The proposed rule and its advantages were very similar to the 2008 draft guidance for industry: that manufacturers of innovative products, such as cell and gene therapy products, as well as manufacturers of currently approved products, may benefit from sterility test methods with rapid and advanced detection capabilities.

The proposed rule also stated that advances in technology (in recent years) have allowed the development of new sterility test methods that yield accurate and reliable test results in less time and with less operator intervention than the currently prescribed methods. Some examples of novel methods with the potential to detect viable contaminating microorganisms that the FDA identified included ATP bioluminescence, chemiluminescence and carbon dioxide head space measurement. Therefore, the proposed rule was not limited to the use of growth-based RMMs, but was now promoting the potential to use non-growth-based RMMs as an alternative to the compendial sterility test.

To summarise, the FDA proposed to amend 21 CFR 610.12 to promote improvement and innovation in the development of sterility test methods, to address the challenges of novel products that may be introduced to the market in the future and to potentially enhance sterility testing of currently approved products. This proposed revision would provide manufacturers the flexibility to take advantage of modern methods as they become available, provided that these methods meet certain criteria.

With respect to validation, USP General Information Chapter <1223>, 'Validation of Alternative Microbiological Methods', was also referenced. Validation of a microbiological method is the process by which it is experimentally established that the performance characteristics of the method meet the requirements for the intended application. For sterility testing, this means that the test can consistently detect the presence of viable contaminating microorganisms.

FDA proposed to eliminate the prescribed sterility test methods found in 21 CFR 610.12 and instead allow the use of sterility test methods that are validated in accordance with established protocols to be capable of reliably detecting viable microorganisms that may be in the test sample. If an established USP compendial sterility test method is used, a manufacturer must verify that this established method is suitable for application to the specific product; however, FDA considered established USP compendial sterility test methods to already have been validated using an established validation protocol, so their accuracy, specificity, and reproducibility need not be re-established to fulfil the proposed validation requirement. In contrast, novel methods and any methods that deviate from the USP compendial sterility test methods would require a detailed validation. For example, when validating non-culture-based methods, the feasibility of identifying microorganisms from a contaminated sample should be evaluated. And if a method does not have the capability to identify microorganisms to the species level, the validation protocol should require that an additional method for species identification be utilised for investigation of detected contaminants. Next, the test organisms selected should reflect organisms that could be found in the product, process or manufacturing environment. Finally, the validation study design should contain the appropriate controls to evaluate the product sample's potential to generate false positive and false negative results. Validation of the sterility test should be performed on all new products, and repeated whenever there are changes in the test method that could potentially inhibit or enhance detection of viable contaminating microorganisms.

One year later, the final rule is published

On 3 May 2012, the FDA amended the sterility test requirements for biological products in their Final Rule, 'Amendments to Sterility Test Requirements for Biological Products'. With an

effective date just a few weeks ago (4 June 2012), the rule revises the sterility requirements for most biological products under Title 21 of the CFR, subchapter F, parts 600 through 680 (21 CFR parts 600 through 680) and is intended to promote improvement and innovation in the development of sterility test methods by allowing manufacturers the flexibility needed for sterility testing of some novel products that may be introduced to the market, enhancing sterility testing of currently approved products, and encouraging manufacturers to utilise scientific and technological advances in sterility test methods as they become available.

Many changes have been put in place. For example, the Final Rule:

- eliminates specified sterility test methods, culture media formulae and culture media test requirements, such as incubation conditions (time and temperature) and visual examination requirements
- eliminates specified membrane filtration procedure requirements for certain products
- eliminates specified sterility test requirements for most bulk material
- modifies the repeat sterility test requirements, so that repeat tests will occur only once for each lot, if due to laboratory error or faulty materials
- replaces the sample size or amount requirement with a requirement that the sample be appropriate to the material being tested
- replaces the storage and maintenance requirements for cultures of test organisms used to determine the 'growth-promoting qualities' of culture media
- requires that the sterility test be appropriate to the material being tested such that the material does not interfere with or otherwise hinder the test

The Final Rule also provides very specific guidance when it comes to RMMs, especially as they relate to validation. For example, a novel method is required to be validated in accordance with an established protocol to demonstrate that the test is capable of consistently detecting the presence of viable microorganisms. Additionally, method validation is a well-recognised activity and can be performed without comparison to a 'referee' test method. Specifically, there is no single 'referee' test method that would work for all products and that some novel methods cannot be easily compared to culture-based methods such as USP Chapter <71> because these testing methods do not measure microbial growth.

The Final Rule also provides definitions and expectations for testing criteria that should be considered during validation:

- The Limit of Detection reflects the lowest number of microorganisms that can be detected by the method in a sample matrix. This is necessary to define what is considered contaminated
- Specificity is the ability of the test method to detect a range of organisms necessary for the method to be suitable for its intended use. This is demonstrated by challenging the sterility test with a panel of relevant organisms in the sample matrix
- Ruggedness is the degree of reproducibility of results obtained by analysis of the same sample under a variety of normal test conditions, such as different analysts, different instruments, and different reagent lots
- Robustness is the capacity of the test method to remain unaffected by small, but deliberate, variations in method parameters, such as changes in reagent concentration or incubation temperatures

Next, the Final Rule provides additional comment on the use of non-growth-based RMMs. For example, the feasibility of identifying microorganisms from a contaminated sample should be evaluated during validation. If a method does not have the capability to identify microorganisms to the species level, the validation protocol should require that an additional method for species identification be utilised for investigation of detected contaminants. Second, the validation study design should contain the appropriate controls to evaluate the product sample's potential to generate false-positive and false negative results. Third, written procedures must include the composition of test components, test parameters, including the acceptance criteria and the controls used to verify the test method's ability to consistently detect the presence of viable contaminating microorganisms. Finally, the volume of test material that results in a dilution of the product should not inhibit or otherwise hinder the detection of viable contaminating microorganisms.

Lastly, the Final Rule specifies that a manufacturer who desires to utilise an alternate sterility test method other than the one approved in its BLA must submit a BLA supplement in accordance with 21 CFR 601.12(b).

Summary

It is obvious that sweeping changes to the finished product sterility test for biologics have been put in place, and I applaud the FDA for thinking outside the box in providing guidance on novel microbiological technologies for this purpose. I am also encouraged with companies that manufacture the types of products, which are covered by the Final Rule, who have already validated and implemented RMMs as alternatives to the compendial sterility test. These changes will certainly encourage others in the industry to adopt rapid methods, not only for sterility testing, but also for all other microbiological applications that we are currently required to perform.

BIOGRAPHY

Dr. Michael J. Miller is an internationally recognised microbiologist and subject matter expert in pharmaceutical microbiology and the design, validation and implementation of rapid microbiological methods. He is currently the President of Microbiology Consultants, LLC (microbiologyconsultants.com). Over the course of approximately 25 years, he has held numerous R&D, manufacturing, quality, and consulting and business development leadership roles at Johnson & Johnson, Eli Lilly and Company, Bausch & Lomb, and Pharmaceutical Systems, Inc. In his current role, Dr. Miller consults with multinational companies in providing technical, quality and regulatory solutions in support of RMMs, sterile and non-sterile pharmaceutical manufacturing, contamination control, isolator technology, validation and microbiological PAT. He also provides comprehensive training for his clients in the areas of rapid method validation and implementation.

Dr. Miller has authored more than 100 technical publications and presentations in the areas of rapid microbiological methods, PAT, ophthalmics, disinfection and sterilisation, is the editor of PDA's *Encyclopedia of Rapid Microbiological Methods*, and is the owner of rapidmicromethods.com, a website dedicated to the advancement of rapid methods. He currently serves on the editorial board for European Pharmaceutical Review, is co-chairing the revision of PDA Technical Report #33: *Evaluation, Validation and Implementation of New Microbiological Testing Methods*, and routinely provides RMM training programs for the industry and professional organisations worldwide.

Dr. Miller holds a PhD in Microbiology and Biochemistry from Georgia State University (GSU), a B.A. in Anthropology and Sociology from Hobart College, and is currently an adjunct professor at GSU. He was appointed the John Henry Hobart Fellow in Residence for Ethics and Social Justice, awarded PDA's Distinguished Service Award and was named Microbiologist of the Year by the Institute of Validation Technology (IVT).