

## Article 6

# The implementation of rapid microbiological methods

**This is the sixth and final paper in a series of articles on rapid microbiological methods that have appeared in European Pharmaceutical Review during 2010. Over the past year, we have explored the world of rapid microbiological methods (RMMs), focusing on validation strategies, regulatory expectations, and the technical and quality benefits of these novel systems as compared with conventional techniques. It should be obvious by now that RMMs will significantly impact the future of microbiology within the pharmaceutical and biotech industries. But don't just take my word for it.**

Rapid methods are becoming a major focus of discussions at professional microbiology and manufacturing science and technology meetings around the world. Most recently, I attended the 5th Annual PDA Global Conference on Pharmaceutical Microbiology in Washington, DC. End-users, regulators and pharmacopoeial expert committee members provided their experiences and perspectives on the future of RMM implementation. I presented a case study on RMM validation and taught a full day training course on the subject. And when I wasn't on the podium, I was blogging on my website, providing summaries of the RMM presentations as they were happening in real-time. That's what I call rapid! In my sixth and final article in this series, I will offer an overview of the discussions that had taken place.

## **Dr. Ed Tidswell discusses the impact of viable but non-culturable (VBNC) organisms**

Dr. Tidswell presented an excellent overview of viable but non-culturable (VBNC) organisms and their clinical implications and risk mitigation in sterile manufacturing. Of the estimated more than 1.5 million different microbial species, less than 0.1 per cent are known to be culturable.

He stated that media fills, environmental monitoring and finished product sterility testing may actually fail to capture all microorganisms that may be present, due to the use of growth-based, conventional assays. Therefore, Dr. Tidswell recommends the adoption of rapid non-growth based PAT microbiology methods to ensure a risk based, integrated approach to the assurance of asepsis, and to reduce our reliance on traditional sterility testing.

## **New technology and method applications validated and implemented**

Jennifer Gray of Novartis Pharma AG, Switzerland, presented their strategy for validating the Millipore Milliflex Rapid as an alternative ATP bioluminescence RMM to the traditional compendial sterility test. The drivers for a rapid sterility test included the early identification of product contamination events, a reduction of throughput time for sterile drug product release, and to increase the company's level of expertise in the field of rapid microbiological methods. Novartis validated a five day sterility test using 22 heat-stressed cultures (seven ATCC strains and 15 environmental isolates). The cultures were also used to determine the most optimal medium to be used

in the system. The FDA approved comparability protocols outlining the validation strategy for multiple products, EMA approval was obtained in February 2010 and MHRA approval was obtained in May 2010.

Amelia Tait-Kamradt, Pfizer, discussed their assessment of Pall's new GeneDisc system. They conducted a number of studies addressing specificity, limit of detection, ruggedness, robustness and the impact that excipients may have on the ability of the system to detect the presence of indicator or specified microorganisms.

Dr. Geert Verdonk of Merck presented his validation studies using the Charles River Laboratories Endosafe PTS. Dr. Verdonk explored the use of this rapid and portable endotoxin detection system as part of Merck's PAT-RMM program. A variety of validation studies were also presented.

Sara Polson, Accugenix, discussed their use of the Bruker MALDI time-of-flight mass spectrometry (TOF MS) microbial identification system. MALDI TOF MS ionises microbial cells and the resulting particles are separated according to size and charge. The resulting spectral fingerprint can be used for microbial identification.

Kevin Luongo, Pfizer, presented an evaluation of the Millipore Milliflex Quantum Rapid Detection System. He described a method utilising viability staining and enumeration of micro-colonies. The non-fluorescent stain is enzymatically cleaved inside the cell, liberating a fluorescent marker that can be detected by the system. For most organisms evaluated, enumeration of low levels or organisms occurred within 24 hours. Part of his assessment included

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demonstrating that the technology is non-destructive and that staining does not impact microbial viability. This may allow subsequent testing of the micro-colonies that have developed, including microbial identification.

Rounding out the technologies discussions, I presented a strategy and case study on false positive testing using the BioVigilant IMD-A, a real-time active air monitoring technology. Materials normally used in cleanroom and manufacturing environments were tested for their potential for eliciting a false positive response (i.e., a positive biological response when no viable microorganisms are actually present). Based on the data obtained, we discussed approaches to minimise or eliminate the potential for observing false positives with the materials evaluated and when using the IMD-A system.

#### Pharmaceopiea perspectives on RMMs provided by the USP, Ph. Eur. and JP Expert Committees

The Chairs of the USP, Ph. Eur. and JP provided their perspectives on the current and future state of rapid methods and plans for revisions to existing monographs and information chapters.

Dr. James Akers, USP Expert Committee, explained that any new United States Pharmacopoeia referee method must be very broad in application and suitable for use with the vast majority of monograph products. Furthermore, new candidate methods must not be from a patented, single-source technology. It is also critical to be clear on the distinction between quality control release testing versus in-process testing and monograph requirements. Therefore, companies that desire to submit a RMM for inclusion in the USP as a referee test must take these points into consideration. USP 1223 was developed to provide guidance on the implementation / validation of alternative methods and this chapter should be used to support the use of a RMM as an alternative to a compendial test. To clarify, RMMs and alternative methods are already allowed under USP 62, as long as they are appropriately validated. Finally, the USP is looking to the industry to comment on the existing chapter 1223 in order to support future revision processes in this area.

Dr. Han van Doorne, Ph. Eur. Expert Committee, stated that the General Notices section of the European Pharmacopoeia and Chapters

2.6.12 and 2.6.13 state that alternative methods may be used as long as they have been shown to be equivalent to the existing compendial methods. Chapter 2.6.27 states that automated systems may be used for the control of cellular products (e.g., for the daily observation of sterility). A separate chapter on the use of nucleic acid technologies for the detection of Mycoplasma (2.6.7) is also available, and Ph. Eur. 5.1.6 was developed to provide guidance on the validation of alternative microbiological methods. Dr. van Doorne then discussed the committee's plans to revise Chapter 5.1.6. They would like to add more information on Process Analytical Technology (PAT), a better distinction for methods for isolation and detection, and for microbial identification. The examples at the end of the current chapter should be improved and expanded to include the validation of ID methods. However, these examples will not appear in a future revision of the chapter, but rather, it will be published as a separate white paper in PharmEuropa. The future revision of this chapter will also include updates to technologies and applications and a greater explanation of DNA-based methods. Finally, he discussed a survey that was sent to the industry

## Delivering compelling economic benefits to manufacturers

The Growth Direct™ System by Rapid Micro Biosystems automates and accelerates detection of microbial contamination in pharmaceutical manufacturing, delivering compelling economic benefits to manufacturers while improving their quality processes. The Growth Direct System is the first and only automated system that addresses all key microbial QC applications and fits with current regulatory practices, a critical accelerator for adoption.

For over 100 years, the traditional culture method has been the accepted technique for routine pharmaceutical and biological quality control testing. The test is inexpensive, but requires time for the culture to grow enough to be seen by the human eye. During this time, product could be waiting in quarantine, or production could be stopped, waiting for a component to

pass inspection. This idle time in manufacturing can cost millions of dollars.

Automated, non-destructive analysis through the Growth Direct eliminates error-prone manual steps and saves labor. Rapid detection of contamination enables manufacturers to reduce lower inventory carrying costs, shorten manufacturing cycle time, and reduce product losses, delivering significant cost savings and increased operational efficiency. Integration to LIMS ensures data consistency and standardization.

Rapid Micro Biosystems, headquartered in Bedford, Massachusetts, provides innovative products for faster detection of microbial contamination in the manufacture of pharmaceutical, biotechnology, and personal care products.



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asking what companies would like to see in a revision of Chapter 5.1.6. Questions included the following: what applications have been approved for use with RMMs, do you use RMMs for testing other than batch release, would you favour more validation examples, what are the strengths and weaknesses of the existing chapter, did the chapter facilitate applications to regulatory bodies, do you consider the chapter example (Annex) useful, and what compendial methods have been replaced by a RMM.

Dr. Tsuguo Sasaki, Japanese Pharmacopoeia Expert Committee, PMDA, described two new RMM chapters that are now part of the Japanese Pharmacopoeia. These include Chapter 22 (Rapid identification of microorganisms based on molecular biological methods) and Chapter 33 (Rapid enumeration of bacteria based on a fluorescence staining method). He then provided some examples of where RMM validations were not at a level that was accepted by the Pharmacopoeia. For example, an ophthalmic manufacturer submitted a validation package for a RMM with a shortened sterility test (two weeks for the compendial method to a one week incubation followed by ATP bioluminescence technology). The company validated the system using soybean casein digest medium instead of a medium that would recover more stressful or injured microbes. For this reason, the submission was not approved. Dr. Sasaki then presented data demonstrating that micro-colonies of a particular organism developed on R2A medium but not on TSA (this is one reason why the original submission was rejected). Therefore, the company would be required to go back and investigate the most appropriate media for this purpose.

### Global regulators provide guidance on validation and implementation expectations

Vivian Christ, Australian TGA first reviewed some of the policies and guidance that they follow with regards to RMMs. The TGA utilises relevant sections in the Ph. Eur. and BP in that these compendia allow for the validation alternate methods. They also rely on the validation guidance from USP 1223, Ph. Eur. 5.1.6, PDA TR #33, and ISO 17025 (validation of non-standard methods), to name a few. From the legislative perspective, the TGA turns to the TGA GMPs, which allows for other acceptable methods as long as they are shown to be equivalent to those in the GMP guide, as well as Annex 11 (computer

validation) and Annex 15 (IQ, OQ, PQ). However, unlike other regulatory agencies, such as the FDA, the TGA only 'quietly' embraces new technologies but they have not come out with a formal statement or policy.

FDA's Dr. David Hussong (CDER) stated that RMMs are very important for meeting Quality by Design (QbD) principles, smart processing and PAT. CDER actively encourages the use of new technologies, and the regulatory mechanisms for implementation of RMMs are evolving. For QbD, ongoing analysis of your processes is expected, and continuous improvement strategies should be utilised, in order to facilitate defining and revision of your design space and understanding what changes in your processes are acceptable. For RMMs, current policy provides for the use of comparability protocols and a number of post-approval change strategies, including prior-approval supplements, annual reports and Special Reports.

Dr. Rajesh Gupta (FDA, CBER) discussed the use of RMMs as an alternative for sterility testing for biologics. From a manufacturing perspective, RMMs can provide faster resolution of process problems, screening of raw materials and implementation of corrective actions. Some biologics have a very short shelf life (e.g., less than 14 days, which is the timing for the incubation phase of the sterility test), are manufactured in small quantities, and may be immediately required for emergency use (e.g., pandemic vaccines). CBER's considerations for a RMM for biologics are viewed on a case-by-case situation depending on the product. The RMM should preferably be a non-destructive technology (the expectation is that a sterility test contaminant can be identified), or use the same / comparable technology as the current methods i.e., growth-based. The RMM should also be shown to detect VBNC organisms.

Finally, Dr. Tsuguo Sasaki, Japanese PMDA, provided his views on the use of RMMs in Japan. The PMDA will work with companies in the development of RMM strategies for use in Japan. For example, in a number of cases, it may not be possible to detect microorganisms in stressed environments, such as purified water and RO test samples. Therefore, it is hoped that more rapid and continuous monitoring methods be developed for microorganism detection in pharmaceutical-grade water systems. Regardless of the technology, the PMDA will follow a similar strategy for reviewing RMM validation submissions as the US FDA does.

### Series conclusion

As you can tell from the current article, the interest in RMMs, either by end-users, regulators or the pharmacopoeia, has reached an appreciable level, and that the implementation of rapid methods has gained the momentum this author has been anxiously waiting for. We no longer need to be trapped in the 19th Century. It is time to take the lead and become technology innovators, just as Angelina Hesse did when she introduced the use of agar, more than 125 years ago. If she were here today, she would be very proud of the direction we are now taking. The future is here, so let's get rapid!

In closing, this has been an exciting yearlong project and I do hope that I have provided you with the necessary tools to explore, validate and implement RMMs in your manufacturing facilities and microbiology laboratories. I will continue to blog on my website, <http://rapidmicromethods.com>, in order to keep you updated on the latest and greatest developments in the world of rapid methods.



#### Dr. Michael J. Miller

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 (<http://microbiologyconsultants.com>). In this role, he is responsible for providing scientific, quality, regulatory and business solutions for the pharmaceutical industry and suppliers of new microbiology technologies. Over the past 22 years, Dr. Miller has held numerous R&D, manufacturing, quality, consulting and business development leadership roles at Johnson & Johnson, Eli Lilly and Company, Bausch & Lomb, and Pharmaceutical Systems, Inc.

Dr. Miller has authored over 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 <http://rapidmicromethods.com>, a new website dedicated to the advancement of RMMs. He currently serves on a number of PDA's programme and publication committees and advisory boards, and is co-chairing the revision of PDA Technical Report #33: *Evaluation, Validation and Implementation of New Microbiological Testing Methods*.

Dr. Miller holds a Ph.D. 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).