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Letter from the Editor

Welcome to the first issue of the 2013 *PMF Newsletter*.

In this issue, Dr. Michael Miller discusses the current state of Rapid Microbiological Methods (RMMs). His discussion includes Quality, regulatory and validation considerations. He expertly dispels common misperceptions about implementation of RMMs, and provides concrete guidance for their implementation.

Dr. Tim Sandle presents an article addressing the variability of the *Limulus* amebocyte lysate (LAL) assay. This compendial test for bacterial endotoxin has several aspects of inherent variability. Dr. Sandle discusses each area, and provides recommendations for statistical analyses. A follow-up article to this will be presented in a future issue of the *Newsletter*.

We are always interested in your input and concerns. Please let us know if there are articles or topics you would like to see addressed in this newsletter.

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The Current State of Rapid Microbiological Methods

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Introduction

During the past 20 years, the field of alternative and rapid microbiological methods (RMMs) has gained momentum as an area of research and application across a number of technology sectors. In fact, much of the development of new systems for the detection and identification of microorganisms has been driven by consumer and patient needs within the food, beverage, environmental and clinical or health care industries. And recent advances in rapid technologies have also encouraged the pharmaceutical and biopharmaceutical industries to validate and implement RMMs in place of their traditional microbiology methods within QC/QA labs and on the manufacturing floor. In fact, companies are now utilizing RMMs for finished product, in-process and raw material bioburden analyses, sterility testing, environmental monitoring, pharmaceutical grade water testing, endotoxin analysis, microbial identification and the detection of Mycoplasma.

Many rapid microbiological method technologies provide more sensitive, accurate, precise, and reproducible test results when compared with conventional, growth-based methods. Furthermore, they may be fully automated, offer increased sample throughput, operate in a continuous data-collecting mode, provide significantly reduced time-to-result (e.g., from days or weeks to hours or minutes), and for some RMM platforms, obtain results in real-time. These methods have also been shown to detect slow-growers and/or viable but non-cultural microorganisms as compared with standard methods used today. Most importantly, a firm that implements a RMM in support of sterile or non-sterile manufacturing processes may realize significant operational efficiencies during the monitoring and controlling of critical process parameters, reducing or eliminating process variability, and reducing the risk to patients. Additional benefits may include the elimination of off-line assays and a reduction in laboratory overhead and headcount, lower inventories (raw material, in-process material, and finished product), a reduction in warehousing space, and a decrease in repeat testing, deviations, out-of-specification investigations, reprocessing or lot rejection.

But before purchasing a RMM, there are a number of technical, quality, business and regulatory due diligence activities that should be considered. As an example, one of the most important aspects in determining the most appropriate RMM for an intended application is to understand your technical or method requirements, the benefits and limitations of available systems, compatibility with the test sample or product, in addition to quality expectations that the method must meet. The latter may include a certain level of sensitivity or limit of detection/quantification, a required sample size and/or the types of microorganisms that should be detected, enumerated or identified.

Current rapid method technologies can detect the presence of diverse types of microorganisms or a specific microbial species (qualitative RMMs), enumerate the number of microorganisms present in a sample (quantitative RMMs), and/or can identify microbial cultures to the genus, species and sub-species levels (identification RMMs). The manner in which microorganisms are detected, quantified or identified will be dependent on the

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specific technology, instrumentation employed, and scientific principle by which the RMM is based upon.

For example, growth-based technologies rely on the measurement of biochemical or physiological parameters that reflect the growth of microorganisms. These types of systems require the organisms in a sample to proliferate, either on a solid or liquid medium, in order to be detected and/or quantified.

Viability-based systems use viability stains and laser excitation for the detection and quantification of microorganisms without the need for cellular growth. Flow cytometry and solid-phase cytometry technologies are examples.

Cellular component-based RMMs rely on the detection and analysis of specific portions of the microbial cell, including ATP, endotoxin, proteins and surface macromolecules.

Optical spectroscopy methods utilize light scattering and other optical techniques to detect, enumerate and identify microorganisms. Real-time analysis with Miie scattering and Raman spectroscopy are now possible.

Nucleic acid amplification-based technologies employ a variety of scientific principles, including PCR-DNA amplification, RNA-based reverse-transcriptase amplification, 16S rRNA typing, gene sequencing and other novel techniques.

Finally, Micro-Electrical-Mechanical Systems (MEMS) utilize microarrays, biosensors, Lab-On-A-Chip or micro-fluidic systems, and nanotechnology, all which provide miniaturized technology platforms as compared with conventional, bench-top instrumentation.

As an end-user of RMMs, ***you*** will be responsible for understanding the technologies that are currently available and to develop a robust and defendable validation and implementation plan that will be acceptable not only to your internal quality organization, but to regulatory authorities as well, when appropriate.

The purpose of this article is to provide you with a summary of RMMs, and what you should consider from a technical, quality, business and regulatory perspective. We have already introduced the principles behind qualitative, quantitative and identifica-

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Variability and the LAL assay

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Introduction

The *Limulus* amebocyte lysate (LAL) assay is the compendia test for the examination of bacterial endotoxin in pharmaceutical products, as well as intermediates, medical devices, and in water (as described in USP chapter <85> and European Pharmacopeia monograph 2.6.14). For large volume parenteral products the LAL test is normally a mandatory test for finished product release (Baines, 2000).

With biological tests all measurements susceptible to variations in analytical conditions should be suitably controlled as far as is practicable. Here the LAL assay has a relatively high level of variability even for a biological assay (Williams, 2007). This variation is due to three principle factors: reagents, the product tested and issues inherent to the method (McCullough and Weider-Loeven, 1992).

This article examines some of the reasons for this variation in relation to the test and the test reagents. The article also examines the coefficient of variation, which is one way to examine for test variation and to allow the laboratory supervisor to take action. Coefficient of variation is measure of precision. The precision of an analytical procedure is the degree of agreement among individual test results (or, in assay terminology, the closeness of individual measures of an analyte when the procedure is applied repeatedly to multiple aliquots of a single, homogenous volume of the biological matrix). It is therefore a useful and important part of quality control.

Part A: Reasons for LAL test variability

As indicated above there are a number of factors which cause LAL test variability. These are outlined in this section of the article.

LAL Reagent

The LAL reagent is a contributor to assay variation. It is noted that the manufacturing processes of different lysate suppliers vary; therefore the list below is somewhat generalized. Variability is for the following reasons:

- The LAL reagent (lysate of the horseshoe crab *Limulus polyphemus*) is of biological origin. It is a complex mixture of enzymes and co-factors. The extract is relatively crude mixture and is not a single purified enzyme. This means that the enzyme activity cannot be determined exactly for each lot of lysate manufactured.
- The manufacturing process also includes the addition of buffers and detergents which contribute a further source of variability.
- The enzymatic activity of each lot of LAL is assessed by the manufacturer using Reference Standard Endotoxin (RSE, supplied by the FDA). The LAL sensitivity is assessed by performing a 2-fold dilution series. This dilution series contributes to the variation.
- The RSE used to characterise the lysate is not readily available to all test laboratories because of its rarity and cost. Laboratories normally use Control Standard Endotoxin (CSE). The potency of CSE is determined by the lysate supplier as-

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sessing the CSE against RSE. This adds a further potential level of test variation.

Bacterial Endotoxin

The endotoxin used in the assay can also cause variation. This is because:

- The endotoxin used to prepare the CSE used in laboratories is from a purified strain of Escherichia coli. CSE is presented as a highly purified lipopolysaccharide free of most detectable contaminants (such as proteins). The CSE contains additional stabilising fillers like starch, human serum albumin and Poly(ethylene glycol) (PEG). However, environmental endotoxin is not purified and normally takes the form of a macromolecular complex of lipopolysaccharide, cellular membrane proteins and phospholipids which are shed by Gram-negative bacteria during growth a death. Thus there is variation in assaying environmental endotoxin against purified endotoxin standards (Brandberg, 1996).

  In addition, although the LAL test is ordinarily specific for endotoxin it will detect only the Lipid A portion of the endotoxin molecule which is available to activate the lysate (the activation of the clotting cascade, the Factor C pathway, is described below) (Moser, 2009: 2). The Lipid-A portion of the endotoxin molecule may form aggregates which are not fully dispersed and therefore not homogenous enough to allow for accurate total measurement.

  Thus a sample which detects endotoxin may not show all of the endotoxin in the sample, for this depends upon the amount of Lipid-A available. Therefore, samples which detect endotoxin may be underestimates. Furthermore, a sample which detects endotoxin may not demonstrate the same level of endotoxin when repeated because the availability of Lipid-A may alter as the chemical nature and stability of the sample alters over time.

- It should also be noted that the toxicity and reactivity of different types of environmental endotoxin differs depending on the biological activity of the lipid-A molecule for different bacterial species.

LAL test variability

The LAL assay is generally considered to have an inherent variability of 50 -200% (or one 2-fold error either side of each endotoxin standard). Varia-

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Past Issues of the *PMF Newsletter* at http://www.microbiologyforum.org/

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tion arises, for kinetic assays, from the slope of the endotoxin standard curve (Kumar, 2007).

Additional variations arise from the following:

- Test tubes;
- Disposable pipette tips;
- Micropipette tips;
  For the above, plastics used in the performance of the BET (e.g. microtiter plates, plastic dilution tubes) are often not made specifically for endotoxin testing.
- Aseptic technique;
- Assay technique;
- Variations in pipetting;
- Variations in preparing control standards;
- Variations in preparing dilutions (which is magnified if the error occurs with the first dilution in the series);
- Dilutions stored over the longer term will show change. Variable factors include temperature, vessel composition, dilution range, volume of the dilution)
- Cross contamination;
- Product or sample interference;
- Sampling containers;
- Sample storage times and temperatures;
- LAL instrument / module variability – different instruments may give different results;
- Presence of endotoxin in product (where endotoxin molecules behave differently or where the availability of lipid-A varies);
- Addition of buffers to stabilize pH? Ancillary solutions may not be free of endotoxin.

Some of the above relate directly to the testing technician (such as making dilutions, pipetting; weighing raw materials and aseptic technique).

Endotoxin Concentrations

Error also increases as the endotoxin concentrations that are used for a standard series become smaller. For example, with a standard curve of 1.0 to 0.1 EU / mL errors of 50-200% will have a lower impact than a standard series of 5.0 to 0.005 EU/mL, based on the smaller value of the last endotoxin concentration in the standard series.

For these reasons the acceptable spike recovery of test controls is 50-200%.

Standard curve linearity

Standard curve consistency is an important feature of the LAL test. A change of only 1% in y-intercept for a linear standard curve can result in a 30- 35% change in endotoxin determination. So, a sample with a known 10 EU/mL can read 13.5 EU/mL, not because of a change in the endotoxin content of the sample, but because of a shift in the y-intercept. An important means to control variability in the turbidimetric LAL test is to keep an eye on the onset (reaction) times. Seemingly small changes in these onset times result in changes to linearity, slope and y-intercept that can have a significant effect on the test result (McCullough, 2008).

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tion technologies, but for a more complete review of the science behind the methods, as well as a comprehensive matrix of RMMs that are commercially available, I encourage you to explore my educational website, http://rapidmicromethods.com, for additional information.

Quality Considerations

Quality risk management (QRM) is an important part of science-based decision making which is essential for the quality management of pharmaceutical manufacturing. The ICH Q9 guideline defines QRM as a systematic process for the assessment, control, communication and review of risk to the quality of drug product across the product lifecycle. Similarly, the FDA's cGMPs for the 21st Century: A Risk-Based Approach, states that using a scientific framework to find ways of mitigating risk while facilitating continuous improvement and innovation in pharmaceutical manufacturing is a key public health objective, and that a new risk-based pharmaceutical quality assessment system will encourage the development of new technologies, such as process analytical technology (PAT), to facilitate continuous manufacturing improvements via implementation of an effective quality system.

Effective monitoring of our manufacturing processes can help to ensure that a state of control is maintained (providing assurance of the continued capability of processes and controls to meet product quality), areas for continual improvement are identified (helping to understand and reduce process variability), process and product understanding is enhanced, and manufacturing agility and efficiencies are realized (by reducing waste and wasteful activities, reduce lead time and increase manufacturing capacity). From a microbiology perspective, we can apply QRM principles in order to design a process to prevent contamination, investigate ways to correct a contamination event, and assess the potential impact of failing results on the patient. This is where RMMs really come into play, because from a microbiological control perspective, we can benefit from utilizing RMMs to:

- design robust processes that prevent contamination,
- ensure that a state of microbial control is maintained,
- develop more effective strategies to correct a contamination problem,
- continually improve our processes and products, and
- assess the potential impact of failing results on the patient.

Regulatory Considerations

RMMs have been implemented by a number of companies around the world, and in some cases, it has been necessary to work directly with the regulatory authorities in order to introduce a RMM as an alternative to an existing microbiology method. This is especially true if the existing method is incorporated in a previously approved regulatory dossier, such as a New Drug Application (NDA) or Marketing Authorisation. However, there are also instances where a formal rapid method submission may not be necessary, and in order to develop an appropriate regulatory strategy for the implementation of RMMs, it is important to fully understand current regulatory expectations.

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There exist a variety of different perspectives on RMM validation and submission strategies, depending on the regulatory body your products are registered with, and/or which local inspectorate is responsible for conducting GMP audits at your manufacturing facilities. For almost 10 years, the U.S. Food and Drug Administration (FDA), the European Medicines Agency (EMA) and other global authorities have accepted and encouraged the use of RMMs, and have provided guidance on how the industry can apply these new technologies in the laboratory and manufacturing environments.

Unfortunately, many in the industry have been hesitant in implementing or even exploring the potential use of RMMs mainly due to a continued perception that the regulators do not accept, understand or embrace RMM technologies. For example, some firms believe that the data coming from a rapid method will put their product at risk, that acceptance levels or specifications will not be met, or that there is no guidance on how to validate these new systems. These myths are simply not true and are not supported, especially in light of the myriad of enablers and guidance that the regulators have provided. Here are some examples of what the regulators have published in support of RMMs:

In 2006, Drs. David Hussong and Robert Mello (New Drug Microbiology Staff at CDER) published a paper entitled, "*Alternative Microbiology Methods and Pharmaceutical Quality Control*." The paper stated the following: "New microbiology methods can offer advantages of speed and precision for solving microbiological problems associated with materials or environmental influences. Neither Corporate economics nor regulatory attitudes should be a barrier to the use of new testing technologies or different measurement parameters. In fact, if we are to increase our understanding of quality-based products and processes, then quality by design principles and risk analysis methods must be extended to the development of new microbiological technologies. This approach will drive process engineering to yield real, measurable gains in microbiological product quality assurance."

Dr. Bryan Riley, New Drug Microbiology Staff at CDER, published a 2004 paper entitled, "*Rapid Microbiology Methods in the Pharmaceutical Industry*." Dr. Riley wrote, "The use of rapid microbiology methods by the pharmaceutical industry should offer many advantages. Receiving microbiology test results sooner will provide for better control and understanding of the manufacturing process via faster feedback. Appropriate validation of rapid microbiology methods is necessary to ensure that the test is suitable for its intended purpose. However, it should be noted that the existing traditional microbiological test methods leave a lot of room for improvement. Therefore, it is not necessary to demonstrate that a new rapid method is flawless, only that it is not inferior to the current method, and will thereby provide equivalent assurance of microbial quality. Current FDA initiatives (i.e., PAT and GMPs for the 21st Century) should help assure industry of the agency's understanding of the potential importance of rapid microbiology methods. These initiatives should also

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convince industry that FDA will assess rapid methods scientifically and not place undue regulatory burdens on firms interested in using these methods. There are many exciting potential uses for rapid microbiology methods in the pharmaceutical manufacturing process, and industry should not feel that FDA will be a hindrance to the appropriate use of these methods."

During the 2009 PDA European Conference on Implementing RMMs, Dr. Riccardo Luigetti (Scientific Administrator at EMA's CHMP/CVMP Quality Working Party (QWP) and member of the EMA PAT Team) stated that RMMs clearly have the potential to be used to support Quality by Design (QbD), and that the introduction of such methods are supported by the EU regulatory competent authorities. During the same conference, Paul Hargreaves (Technical Manager, U.K. Medicines and Healthcare Products Regulatory Agency; MHRA) viewed the implementation of alternative microbiology methods as a positive step in improving the quality of medicines and patient safety, and has actively encouraged the industry to implement these technologies for many years.

More specifically, the following enablers are now in place to help companies develop a meaningful validation and implementation plan:

The U.S. FDA

- Validate RMMs according to any of the current guidance or your own strategy, as long as it can be defended. This can include USP Chapter <1223>, Ph. Eur. 5.1.6 and/or PDA Technical Report #33.
- Submissions may include the use of a comparability protocol, in which your proposed validation plan can be reviewed and approved for use, and the results of the validation (and not the actual data) is submitted in a brief Special Report. Under this strategy, any future CMC changes covered under the approved comparability protocol can be made without the need for additional approvals.
- A reduced reporting category can also be utilized when implementing a RMM, such as a Changes Being Effected, or CBE-0.
- A Research Exemption can be employed while the RMM is being validated, in which GMP decisions, including batch release, are based on the current approved validated methods, and not the data generated by the RMM under investigation.

The EMA

- Validate RMMs according to Ph. Eur. 5.1.6. Alternatively, the chapter does not need to be followed exactly, as long as all deviations

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Beta-glucans

The LAL test is not always specific for bacterial endotoxin and may sometimes detect glucans, arising from cellulose or from fungi (Moser, 2009: 3). β-Glucans are soluble polysaccharides of glucose that can be produced by many prokaryotic and eukaryotic organisms. Common sources of glucans in pharmaceutical manufacturing are filters made from cellulose materials, plant-derived raw materials, cotton-containing enclosures, sugars, naturally-derived raw materials, and cellulose products (such as sponges or filters). A second source is fungi (or yeast hydrolysate) (Pearson *et al*, 1984).

The presence of (1→3)-ß-D-glucans can give a falsely higher reading in the Limulus amebocyte lysate (LAL) assay for endotoxins (so termed 'false positives'). The presence of glucans also has a tendency to cause test interference (that is when the test indicates the presence of endotoxin when none is in fact present). Therefore, interference from glucans could produce and Out-Of-Specification result. Some manufactures of lysate used in the LAL assay produce variants which will not detect glucan (that is, they are endotoxin specific).

Part B: Assessing variation through the coefficient of variation

The coefficient of variation (C.V.) is a measure of test precision, between two or more replicates. The use of the coefficient of variation is, however, not straightforward because statistics text books describe different methods to determine it. Moreover, for the LAL test there are different parameters that can be used to calculate the C.V., such as actual endotoxin results or measurement of optical density. To complicate things further, there are different limits that can be applied for what is an acceptable range for the coefficient of variation. These difficulties are explored below.

What is the coefficient of variation (C.V.)?

The C.V. is the standard deviation expressed as a percentage of the mean. It allows a comparison of the variability to be made between concentrations through the production of a dimensionless number (i.e. a number with no units). As the mean between different samples increases, then the CV is a measure that accounts for this variability. It is converted into a percentage by multiplying the obtained number by 100 to produce the % C.V. (Rosner, 1990: 23).

How is the %C.V. calculated?

There are different approaches to the calculation of C.V.s. These relate to different ways of calculating the standard deviation.

Different approaches are based on the way of calculating the standard deviation. This can be a standard deviation which measures the entire population (n) or one that measures a representative population (n-1). The ACC approach uses a population of n-1 because the volume of endotoxin used in the LAL test only represents a small volume of the original solution (the reader should note that there is a difference between US and European terminology for a population subset. In the US the term 'population' is used whereas in Europe it is more common to use the term 'sample').

Once the approach for calculating the standard deviation has been adopted, the C.V. becomes expressed as the ratio between the standard deviation and the mean. From this, the %C.V. can be calculated, thus:

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100% x (standard deviation / arithmetic mean)

What is the value of measuring the %C.V. for LAL testing?

The C.V. is useful in comparing means and determining if they differ substantially.

The lower the %C.V. the closer the level of precision between the different test replicates is (the 'scatter' from the mean is relatively small).
The higher the %C.V. the weaker the level of precision between the different test replicates is (the 'scatter' from the mean is relatively large).

What is the acceptance limit for %C.V.?

According to Richardson and Novitsky, there is no absolute C.V. that will determine a 'good' or 'bad' LAL test.

Studies have shown that C.V. values calculated on measurements of EU/mL typically increase for lower concentrations of endotoxin, in that values of less than 10% can often be obtained for higher concentrations of endotoxin, whereas values of between 10 and 30% are obtained for lower values of endotoxin (typically towards the end of the standard curve and close to the limit of detection).

It should be noted at this point that different LAL suppliers have different acceptance criteria for the LAL test and calculate their %C.V.s, through software packages, in different ways. Some, for example, calculate the coefficient of variation based on the results of obtained in Endotoxin Units per millilitre (EU/mL); whereas others calculate coefficient of variations based on the sample onset times (in milliabsorbance units).

Whichever cut-off value is applied, based on the available literature (Lindsay *et al*, 1989; Rafa *et al*, 1999), tests that are above 25% are atypical and require examination.

Summary

This article has considered some of the sources of variation which affect the LAL test. These are important for the laboratory user to understand, especially for the design of the assay and with the investigation of test anomalies. The article has also considered one key measure of variability: the coefficient of variation. This is an important check for the laboratory supervisor to include when reviewing test results. One final and important aspect of LAL test variation is analyst error. This will be assessed in a second article which will appear in a future issue of the PMF Newsletter.

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from the guidelines are clearly stated and reasons provided.

- Under the newly revised regulations, you can now group variations under the same Marketing Authorisation such that they can all be assessed at the same time. Additionally, you can combine the same variations or group of variations from different Marketing Authorisations and have all of these assessed at the same time under a "work sharing process" or "common assessment". This can include a single RMM technology being used for multiple products.
- The new Scientific Advice procedure now provides opportunities for scientific dialog with regulators.
- The new Post Approval Change Management Protocol provides a similar process for the pre-approval of a validation plan, similar to the FDA's comparability protocol, in addition to the use of a reduced reporting structure. The main difference is that the validation data will be submitted back to the regulators.
- A Quality Expert Report is the document used to convey the information from the RMM validation studies.

The Australian TGA, Japanese PMDA and Rest of World

- Both of these regulatory authorities follow similar processes as the FDA and EMA do.
- The remaining regulators (rest of the world) may not have formal guidance on the validation and use of RMMs; therefore, you should discuss your plans with each, as appropriate.

Regardless of the regulatory agency, it is important that you discuss your plans in the early phases of RMM planning, to ensure that all parties are "are on the same page."

Validation Considerations

Method validation is the process used to confirm that an analytical procedure employed for a specific test is reliable, reproducible and suitable for its intended purpose. All analytical methods need to be validated prior to their introduction into routine use, and this is especially true for novel technology platforms, such as RMMs.

Because many RMM technologies consist of a combination of instrumentation, software, consumables and reagents, in addition to specific detection, quantitative or identification methodologies, it is important to develop a comprehensive and holistic approach to the validation process to ensure that the entire RMM system is suitable for its intended use. You are encouraged to follow the guidance as outlined in USP <1223>, Ph. Eur. 5.1.6 and/or PDA Technical Report #33. However, you should also know that all three of these documents are currently under revision, and that slight changes to the existing recommendations may change. Of note is the PDA TR33, in which an expanded validation section, including the use of statistics, will be provided (it is anticipated that the TR will be available this year).

In general, the following phases are to be completed during the validation of a RMM. Due to space limitations, it is not possible to provide a thorough review of what is expected in this article; however, additional information may be found on the Validation section at http://rapidmicromethods.com.

- Risk Assessment
- Validation Master Plan (VMP)
- User Requirements Specifications (URS)
- Design Qualification (DQ)

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- Supplier Assessment/Audit
- Functional Design Specifications (FDS)
- Requirements Traceability Matrix (RTM)
- Training and Standard Operating Procedures (SOPs)
- The Test Plan
  - ◊ Factory Acceptance and Site Acceptance Testing, only when applicable
  - ◊ Installation Qualification (IQ)
  - ◊ Operational Qualification (OQ). This is usually where specific validation criteria are tested, including accuracy, precision, limit of detection, limit of quantification, linearity, range, specificity, robustness and ruggedness.
  - ◊ Performance Qualification (PQ). This is usually when equivalency studies are performed, in which a side-by-side comparison is conducted between the RMM and the reference or existing method, using the actual test sample, such as finished product or in-process material.
- Validation Summary Report

Summary

RMMs offer new solutions for the detection, enumeration and identification of microorganisms. It is your responsibility to understand your firm's current methods, look at opportunities for improvements, identify the appropriate RMM for the intended application, and validate the method for routine use. Guidance that is recommended by the regulators, what is provided in the public domain, and what I have communicated in this article, should present you with the quality and technology tools that are necessary to implement RMMs for enhanced contamination control and meeting the spirit of Quality by Design and continuous process and product improvement.

About the Author:

Dr. Michael J. Miller is an internationally recognized 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). Over the course of 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 sterilization, is the editor of PDA's Encyclopedia of Rapid Microbiological Methods*, and is the owner of http://rapidmicromethods.com, a website dedicated to the advancement of rapid methods, and where much of this article was adapted from. He currently serves on the editorial board for European Pharmaceutical Review, is 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 organizations worldwide.*

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).

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