

Compendial Requirements for Automated Microbiological Method Validation: The Role of USP Chapter <16> “Automated Methods of Analysis” and the Proposed Chapter <1058> “Analytical Instrument Qualification”

David Jones, Genomic Profiling Systems, and Scott Sutton, Vectech Pharmaceutical Consultants

Pharmacopeial microbiological tests detect and/or enumerate microbes that replicate in the presence of microbiological media. The challenge presented by adopting modern rapid microbiological methods is determining whether or not they represent the automation of a current compendial method or if they represent an “alternative” to a compendial method. The answer impacts how a firm should qualify and validate the new rapid method and may provide an opportunity for a more streamlined validation protocol.

The difference is critical because of two chapters in the U.S. Pharmacopeia. USP Chapter <16> “Automated Methods of Analysis” has been very useful to the chemistry community since its introduction in 1975. It provides a means to qualify new automation methods without engendering the full burden of a complete qualification/validation process, as described in USP Chapter <1225> “Validation of Alternative Methods.” Chapter <16> provides examples of several tests that are amenable to automation in the chemistry laboratory, but does not address microbiological methods. A second consideration in the method validation is instrument qualification. Although a general GMP requirement, instrument qualification studies are not addressed in these chapters, a failing USP is addressing through the recently proposed <1058> “Analytical Instrument Qualification.”¹

With interest in rapid microbiological methods rising, there is a variety of new technologies available to the quality control

(QC) microbiology laboratory that will move the microbiology lab into the 21st century. While many of the more widely discussed rapid methods are based on technologies completely dissimilar to the current pharmacopeial methods (PCR, viable dye, flow cytometry, etc.), several automated microbiological tests rely on traditional microbiological methodologies to detect and count microorganisms. There are several rapid micro systems currently available that automate detection and enumeration of cells replicating to form colonies on plates containing nutrient media. Examples of these technologies are the *QCount* from Spiral Biotech, the *ProtoCol* from MicroBiology International (MI) and the *Growth Direct™ System* from Genomic Profiling Systems (GPS).

The regional compendia are moving forward on the question of rapid microbiological methods. The USP draft chapter <1223> “Validation of Alternative Microbiological Methods”² will be official in August of this year, and the EP chapter 5.1.6 “Alternative Methods for Control of Microbiological Quality”³ is now in force. Both regional compendia recognized the need to provide more appropriate definitions to the accepted validation criteria of accuracy, precision, limit of quantification, etc. This was required as it was recognized early on that the established terms, while appropriate for chemistry, were unworkable in the validation of microbiological assays due to the larger degree of variability in the system.^{3,4} While these “validation guides” are useful, they

assume that the new technology is in fact new and different from the compendial methods.

There is a need to distinguish between “automated compendial” methods and “alternative” tests, as they require different validation approaches. Automated compendial tests differ from alternative microbiological tests in that the automated tests are based on the same methods and principles and measure the same targets as the manual compendial tests. Alternative tests, on the other hand, use distinct methods and principles and measure distinct targets, such as ATP bioluminescence, “fluorescent events,” etc., compared to compendial tests.

It is also worth mentioning that the compendial “method” under review may not actually be the title of the USP chapter. For example, the sterility test can be described as two discrete steps:

- 1) Filtration of the sample
- 2) Examination of the filter for the presence of viable cells.

A “rapid” sterility test will probably have the same design as the compendial test (now harmonized)—20 units of product will be filtered, and the filter will be assayed for viable cells.⁶ The “rapid” part only comes in as you specify the method used to assay for viable cells. Similarly, many quantitative assays do not differ significantly from the compendial method except in the manner of determining the number of cells present. Here there may be more of a concern. The compendial method for enumeration is to grow colonies on or in an agar ►

surface. The colony forming unit (CFU) may arise from one cell or several thousand; it becomes visible only after there are several tens of millions in the colony after replication.

Alternate methods of enumeration that are not based on the CFU are fundamentally different from the compendial method of enumeration used in the microbial limits tests, the antimicrobial efficacy test and others. For example, the AES Chemunex *ScanRDI* method measures the numbers of cells showing esterase enzymatic activity rather than the number of colony forming units—the quantity measured by the compendial methods. Consequently, the targets measured by the *ScanRDI* system can be very different than those measured by the compendial tests, since not all of cells with esterase activity can replicate in the presence of microbiological media⁷.

Automated compendial tests differ from the manual compendial tests only in that some manipulations and/or detection steps are automated. For example, colony counting by GPS' *Growth Direct System*⁸ and the *QCount* from Spiral Biotech uses the same method principles (growth of colonies on an agar surface) and measures the same colonies as do the tests described in several USP chapters. Both the manual and automated approaches enumerate colonies derived from microbes that can replicate on a media support. The automated system, however, uses digital imaging to detect the colonies, in contrast to the manual method in which colonies are detected by eye. The automated imaging is more reproducible and allows faster enumeration times.

For alternative tests, validation must be concerned with demon-

strating that measuring different targets leads to equivalent or better results compared to the compendial methodology. However, USP <16> argues persuasively that an automated test need only demonstrate accuracy and precision. If we allow for the strategy and definitions in the proposed USP Chapter <1223>, application of the approach embodied in USP <16> for validation of automated methods in microbiology should be appropriate. In this approach, once the equipment is qualified, the method need demonstrate only accuracy and precision equivalent to the compendial method.

This does bring up equipment qualification as a concern. The 2005 Pharmacopeial Preview for the proposed USP chapter <1058> "Analytical Instrument Qualification" states:¹

Good Manufacturing Practices (GMP) regulations require companies to establish procedures ensuring the fitness for use of instruments that generate data supporting regulated product testing. However, GMP regulations do not provide definitive guidance for the qualification of analytical instruments.

The chapter's goals are described:

This chapter covers the initial part of the data quality acquisition process (qualification, validation, and verification), defines the roles and responsibilities of those associated with an instrument's qualification, and establishes the essential parameters for performing instrument qualification and a common terminology.

In response to public concerns, USP published a revised draft which presents the opportunity to accept the system suitability test as proof of suitable performance for the PQ portion of the quali-

fication.⁹ This chapter is being finalized for publication. Once finalized, it will serve not only for automated microbiological methods, but all equipment qualification studies.

A major concern with acceptance of alternate microbiological methods is uncertainty over validation and the associated costs. However, the opportunities for these methods to streamline testing is enormous.^{10,11} Clearly the different types of alternate microbiological methods have differing degrees of risk associated with them and should have differing validation burdens. Many of these automated technologies clearly fall in the same philosophical category as was envisioned by USP in the creation of a dedicated chapter describing the validation of an automated, rather than an alternative, method. ☺

References:

- ¹ U.S. Pharmacopeia, "<1058> Analytical Instrument Qualification," *Pharmacopeial Forum*, vol. 31, no. 1 (2005), pp. 233-243.
- ² U.S. Pharmacopeia, "<1223> Validation of Alternative Microbiological Methods," *Pharmacopeial Forum*, vol. 31, no. 5 (2005), pp. 1475-1486.
- ³ European Pharmacopoeia, "5.1.6 Alternative Methods for Control of Microbiological Quality," *PharmEuropa*, Suppl. 5.5, pp 4131-4142.
- ⁴ Sutton, S.V.W., "Validation of Alternative Microbiology Methods for Product Testing: Quantitative and Qualitative Assays," *Pharmaceutical Technology*, vol. 29, no. 4 (2005), pp. 118-122.
- ⁵ Knapp, J.E., et al., "Developing an Information Chapter in the USP to Demonstrate Equivalency in Microbiological Methods," *American Pharmaceutical Review*, vol. 5, no. 2 (2002), pp. 14-19.
- ⁶ Moldenhauer, J. and S.V.W. Sutton, "Towards an Improved Sterility Test," *PDA Journal of Pharmaceutical Science and Technology*, vol. 58, no. 6 (2004), pp. 284-286.

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Next in the process was creation and review of a list of potential materials of construction for the jacket and internal sealing surfaces. Nine potential materials in ten common chemicals were tested for hardness, change in mass, total organic carbon (TOC) extractables, nonvolatile residue (NVR), and small molecule clearance. By individually testing all materials, the team was able to determine which provided the best combination of cleanliness and performance.

The original list of ten items was trimmed to three that met all acceptance criteria. These three materials were then introduced in alpha product samples and subjected to specific product tests. As a result, the team selected the two materials that not only met all acceptance criteria but also maximized performance.

Pellicon® 3 then moved into the detailed development stage. Manufacturing and development engineers, under the guidance of quality engineers, conducted experiments to help understand the contributions and interactions of process variables on the process and final product. One project requirement was to identify critical process parameters. The team conducted numerous designs of experiment (DOE) on isolated process steps to simplify the development process.

One basic building block for a Pellicon® 3 device is a membrane packet consisting of a permeate screen sandwiched between two membranes. Membrane packets are then separated by feed screens and stacked until the correct membrane area is achieved. To increase throughput while improving quality and cleanli-

ness, the project team designed an automated packet assembly machine (APAM) that is fed rolls of membrane and pre-cut screen, which are converted into finished packets. The APAM produces packets that are then in-line and on-line tested before robotically being stacked.

In determining the ideal machine operating conditions, the team conducted several DOEs to understand the impact of all process variables employed in manufacturing the packets.

For example, experiments were developed to measure the impact of each individual variable, as well as combinations of multiple variables, on selected outputs such as packet integrity or thickness (Figure 1, p. 28). Three critical heat sealing parameters were identified. This understanding allowed the engineers to focus on the most critical parameters and to establish appropriate operating specifications that result in a repeatable process that delivers packets of known performance characteristics (Figure 2, p. 28).

The knowledge gained from the DOEs enabled the proactive analysis of critical process parameters and will help in future development projects. Manufacturing engineers will continue to collect data and increase their understanding of the process. These steps will further reduce product variability by enabling modifications to the control methods and/or limits.

Delivering Quality

The final cassette manufacturing processes include design and manufacture to cGMP standards. These include cleaner design and manufacturing environments, use

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⁷ Yvon, P., "Viability-based Technologies: Solid-phase Cytometry Using Chemunex ScanRDI," *Encyclopedia of Rapid Microbiological Methods*, vol. 2, edited by M.J. Miller, pp 291-315 (PDA and DHI Publishing, LLC, 2005).

⁸ London, R., et al., "The Growth Direct Test: A Rapid, Non-destructive Automated System for Microbial Enumeration," *Encyclopedia of Rapid Microbiological Methods*, vol. 2, edited by M.J. Miller, pp 103-135 (PDA and DHI Publishing, LLC, 2005).

⁹ U.S. Pharmacopeia, "<1058> Analytical Instrument Qualification," *Pharmacopeial Forum*, vol. 32, no. 2 (2006), pp:595-605.

¹⁰ Hussong, D. and R. Mello, "Alternative Microbiology Methods and Pharmaceutical Quality Control," *American Pharmaceutical Review*, vol. 9, no. 1 (2006), pp. 62-68.

¹¹ Cundell, A.M., "Opportunities for Rapid Microbial Methods," *European Pharmaceutical Review*, vol. 1 (2006), pp. 64-70

About the Authors

David Jones, PhD, is the Director of Technical Services at Genomic Profiling Systems. He has many years experience with rapid microbiology methods, including time at Wyeth Biopharma where he helped evaluate and validate rapid methods and new technologies to improve laboratory efficiencies.

Scott Sutton, PhD, has had over 20 years of industrial experience with companies such as Bausch and Lomb and Alcon Laboratories before joining Vectech Pharmaceutical Consultants in 2004. Among his various affiliations, Dr. Sutton operates an information source on the internet—The Microbiology Network (<http://www.microbiol.org>) that provides services to microbiology related user's groups—and supports the PMFLIST, an e-mail list devoted to pharmaceutical microbiology (www.microbiol.org/pmflist_info.htm), and the Pharmaceutical Stability Discussion Group (www.microbiol.org/psdglst_info.htm).