

WHITE PAPER



ENHANCE THE DEVELOPMENT OF RAPID MICROBIOLOGICAL METHODS

Molecular-based rapid microbiological methods (RMM) are contemporary technologies used to quickly and sensitively detect, enumerate, and identify microorganisms. Within the past several decades, these methods have gained recognition for their technological applications across a number of scientific fields. In particular, the advancements and associated benefits of RMMs have begun to accelerate the validation and implementation of these technologies in pharmaceutical quality management procedures. To date, these methods have proven useful in a variety of pharmaceutical sterility assays including the routine examination of microbial limit testing, bioburden assessment, environmental testing, raw materials testing, process water testing, sterility testing, and in-process testing.

Though RMMs are considered by much of the scientific community to be an improvement over classical growth-based technologies, a large proportion of the pharmaceutical industry is still hesitant to adopt these methods due to perceived concerns regarding cost, precision, accuracy, and validation. Rather, traditional culture-based methods are predominantly employed in pharmaceutical quality management analyses to detect and enumerate objectionable microorganisms throughout the manufacturing process. Although the growth of microbes on solid surfaces can provide critical information regarding the concentration and type of organism that may be present, the incubation and evaluation period can be quite time intensive, resulting in costly workflow delays. Additionally, not all microbial species can be propagated under artificial growth conditions. This can be problematic as undetected microbial contaminants in medicinal products can seriously impact patient morbidity and mortality. To alleviate these concerns, the pharmaceutical industry should consider the industry-wide implementation of

contemporary molecular-based RMM technologies to ensure the rapid and sensitive detection of microbial contaminants.

Presently, quantitative molecular-detection RMM technologies, such as polymerase chain reaction (PCR) and real-time PCR, are the most common techniques used to detect microorganisms.^{1,2} These technologies provide numerous benefits over traditional culturing methods. For example, in both technologies, universal primer sets can be used to amplify highly conserved regions of the microbial genome, allowing for detection at the species level. In particular, real-time PCR provides for the simultaneous amplification and detection of microorganisms. Further, in contrast to classic cultivation techniques, which can take days, molecular-based RMM technologies detect and identify minute microbial populations within hours. Furthermore, these processes employ a high degree of automation, allowing for improved process control, increased sensitivity, electronic data capture, and fewer repeat tests. Replacing culture-based testing methods with molecular assays may also reduce long-term costs associated with required cultivation materials including segregated laboratory space, media, reagents, and incubators. Such measures will enhance business productivity with fewer product delays and rejections, allowing for quicker product releases.

When evaluating an RMM system for use, a variety of factors must be considered. The functional and analytical aspects of the current microbial assay should be assessed to identify the proper RMM technology required, and the initial cost of the system should be examined to determine essential advantages from repeated use. Once decided upon, the level of automation required by the RMM system must be evaluated to determine training requirements, and each system should be validated for accuracy, limit of quantification, linearity, and range prior to employment. Reference standards

required for each microbial analysis protocol must be identified and obtained from a reliable source, such as ATCC. This latter consideration is of particular importance in molecular-based RMMs as these techniques are very sensitive. To ensure the accuracy of molecular methods, it is essential to employ authenticated nucleic acids as standardized controls.


To enhance molecular-based RMM procedures, ATCC offers a number of purified nucleic acids that allow the pharmaceutical industry to save both the time and expense associated with nucleic acid extraction and purification. Currently, ATCC products consist of nucleic acids extracted from numerous standardized reference strains, including those recommended for various pharmaceutical sterility testing protocols listed in the United States Pharmacopeia.³ Each preparation is isolated under strict aseptic conditions to ensure that cross-contamination does not occur, and preparations are evaluated for purity, integrity, and quality using Picogreen® measurements, Agarose gel electrophoresis, spectrophotometry, and sequencing. In addition to their employment in RMMs, ATCC nucleic acids have proven essential in applications such as the construction of genomic libraries to document contamination events and the development of novel microbial detection assays.


Overall, the pharmaceutical industry will benefit from the change to RMMs, and ATCC nucleic acids are ideally suited as RMM reference standards for the molecular-based microbiological analysis of pharmaceutical products. For more information about ATCC products, please contact ATCC Technical Services at Tech@atcc.org.

REFERENCES

- 1 RMM. *Rapid Micro Methods*. 2012 [cited; Available from: <http://rapidmicromethods.com/>].
- 2 Moldenhauer, J., *Rapid Microbiological Methods and the PAT Initiative BioPharma International* September 15 Issue. 2005.
- 3 Convention, U.S.P., USP35 NF30, 2012: *U. S. Pharmacopoeia National Formulary*. 2012: United States Pharmacopeial Convention.

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