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Application Note

Automating Rapid Microbiological Method (RMM) Validation with Ready-to-Use MicroQuant™ Microbial Reference Materials

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ABSTRACT

Rapid microbiological methods (RMMs) offer numerous advantages over traditional culture-based testing; however, their adoption in pharmaceutical quality control is frequently delayed by the complexity and variability of method validation. A critical bottleneck is the reliable preparation of precisely quantified microbial inocula. Here, we demonstrate how MicroQuant™ quantitative reference microorganisms address this challenge. Across a panel of MicroQuant™ strains representing four compendial-relevant organisms, consistent inoculum control was observed throughout the RMM validation workflow. When integrated with the μ Count3D™ (BioSense Solutions), an automated microbial cell counting system, and the IntuGrow system (IntuBio), a rapid growth-based colony forming units (CFU) detection platform, MicroQuant™ controls exhibited reproducible growth kinetics and quantitative agreement with the compendial plate-count method. These results demonstrate that MicroQuant™ controls support faster, more confident RMM validation in alignment with the United States Pharmacopeia (USP) and European Pharmacopeia (Ph. Eur.).

Introduction

Microbial contamination remains one of the leading causes of pharmaceutical product recalls, with significant consequences for patient safety, regulatory standing, and brand reputation.¹ To mitigate these risks, regulatory authorities require pharmaceutical manufacturers to perform robust microbiological quality control (QC) testing in alignment with guidance defined by global pharmacopeia organizations like the United States Pharmacopeia and European Pharmacopoeia.²

Conventional microbiological QC test methods rely heavily on culture-based techniques like plate counting, which can be labor-intensive, time-consuming, and susceptible to variability arising from operator technique, culture conditions, and differences in microbial growth state. Rapid microbiological methods (RMMs) have emerged as an attractive alternative, offering faster time to results, reduced hands-on time, and improved process control. However, their adoption is often delayed by the complexity of method validation—a requirement of USP Chapter <1223> and Ph. Eur. Chapter 5.1.6.^{3,4}

A critical and often underestimated bottleneck in RMM validation is the preparation of reproducible microbial inocula, which can introduce variability, prolong validation timelines, and necessitate repeat testing. To overcome this limitation and enable more reliable RMM



implementation, laboratories require access to inocula that deliver consistent and quantifiable performance. Unlike traditional overnight culture workflows, which introduce variability from growth phase and operator handling, standardized quantitative reference materials can provide a more reproducible starting point for method validation. MicroQuant™ reference microorganisms were developed to address this need through a ready-to-use, precisely quantitative format that enables rapid inoculum preparation while reducing variability associated with culture expansion and manual enumeration.⁴ By providing a defined microbial input, MicroQuant™ reference materials have the potential to improve inoculum control and support more efficient validation of alternative microbiological methods.⁵⁻⁸

To evaluate the utility of MicroQuant™ for RMM validation, we assessed four compendial-relevant MicroQuant™ reference microorganisms—*Staphylococcus aureus* subsp. *aureus* (ATCC® 6538-HQ-PACK™), *Pseudomonas paraeruginosa* (ATCC® 9027-HQ-PACK™), *Candida albicans* (ATCC® 10231-HQ-PACK™), and *Aspergillus brasiliensis* (ATCC® 16404-HQ-PACK™)—in combination with the µCount3D™ automated microbial cell counting system and the IntuGrow rapid growth-based detection platform. This study examined method comparability against compendial plate counting, as well as workflow efficiency, inoculum control, recovery, linearity, and precision.

Materials and Methods

Reference Microorganisms

Four high-CFU MicroQuant™ products representing compendial-relevant organisms were evaluated as controls for RMM validation with µCount3D™ and IntuGrow. These reference materials included gram-positive and gram-negative bacteria, a yeast-form fungus, and filamentous fungal spores (Table 1). All MicroQuant™ products were retrieved from 2–8°C storage and rehydrated in accordance with their respective Instructions for Use (IFU), available on the ATCC® website.^{9,10}

Table 1: MicroQuant™ reference products evaluated in this study.

ATCC® No.	MicroQuant™ Product
16404-HQ-PACK™	MicroQuant™ <i>Aspergillus brasiliensis</i> , high CFU (Pack of 5)
10231-HQ-PACK™	MicroQuant™ <i>Candida albicans</i> , high CFU (Pack of 5)
6538-HQ-PACK™	MicroQuant™ <i>Staphylococcus aureus</i> subsp. <i>aureus</i> , high CFU (Pack of 5)
9027-HQ-PACK™	MicroQuant™ <i>Pseudomonas paraeruginosa</i> , high CFU (Pack of 5)

Experimental Design

The study consisted of two complementary evaluations (Figures 1 and 2). First, MicroQuant™ reference microorganisms were used to assess the suitability of a rapid microbiological method (RMM) workflow through comparison with the compendial plate count method (Figure 1). Following rehydration, MicroQuant™ suspensions were quantified using the µCount3D™ automated microbial cell counting system and diluted to target inoculum concentrations. The resulting suspensions were analyzed in parallel using the IntuGrow rapid growth-based detection platform and the compendial plate count method to evaluate method comparability, including linearity, recovery, and precision in accordance with USP <1223>.

A second evaluation compared the MicroQuant™ workflow with a conventional culture-based inoculum preparation workflow (Figure 2). In the conventional approach, microorganisms were recovered from frozen stock or agar cultures and expanded through subculture prior to analysis. Performance was compared following inoculum preparation to assess workflow efficiency, inoculum standardization, and variability. This comparison was designed to evaluate the impact of pre-quantified, ready-to-use reference microorganisms on RMM validation workflows.

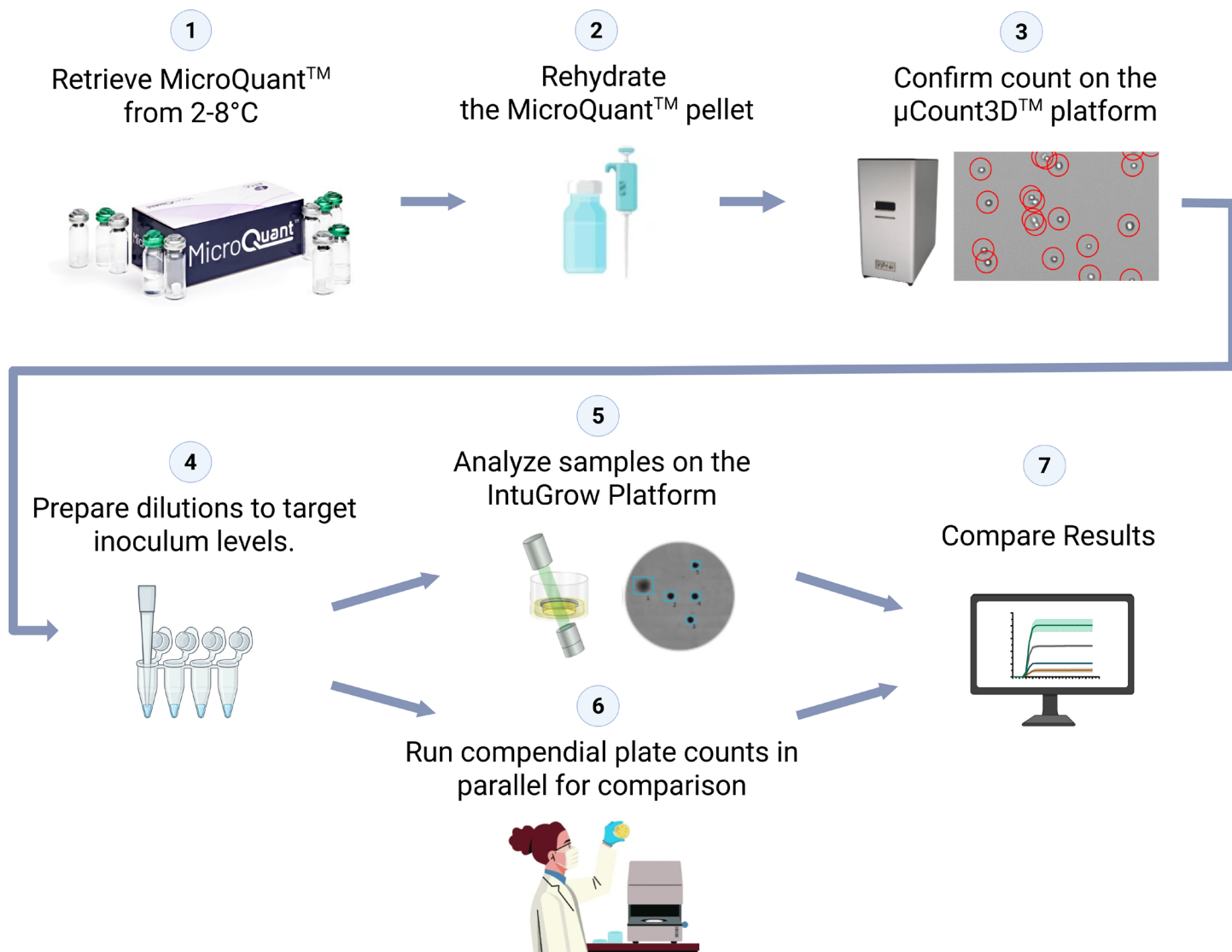


Figure 1: Workflow for RMM validation using MicroQuant™ – Parallel comparison of IntuGrow and the compendial plate count method.

The workflow illustrates a standardized approach for generating and evaluating microbial inocula for method comparability and validation. (1) MicroQuant™ vials are retrieved from 2–8°C storage, followed by (2) rapid rehydration of the MicroQuant™ pellet using the supplied buffer. (3) The starting concentration is confirmed using the μCount3D™ platform (an automated microbial cell counting system) to ensure accurate inoculum input. (4) Serial dilutions are prepared to achieve target inoculum levels. (5) Samples are analyzed using the IntuGrow rapid growth-based detection platform, while (6) parallel enumeration is performed using the compendial plate count method. Finally, (7) results from both methods are compared to assess quantitative agreement and method equivalence in accordance with USP <1223>. The MicroQuant™ workflow uses a prequantified, standardized format with minimal handling, reducing many of the sources of variability associated with conventional overnight culture workflows. Created with BioRender.com.

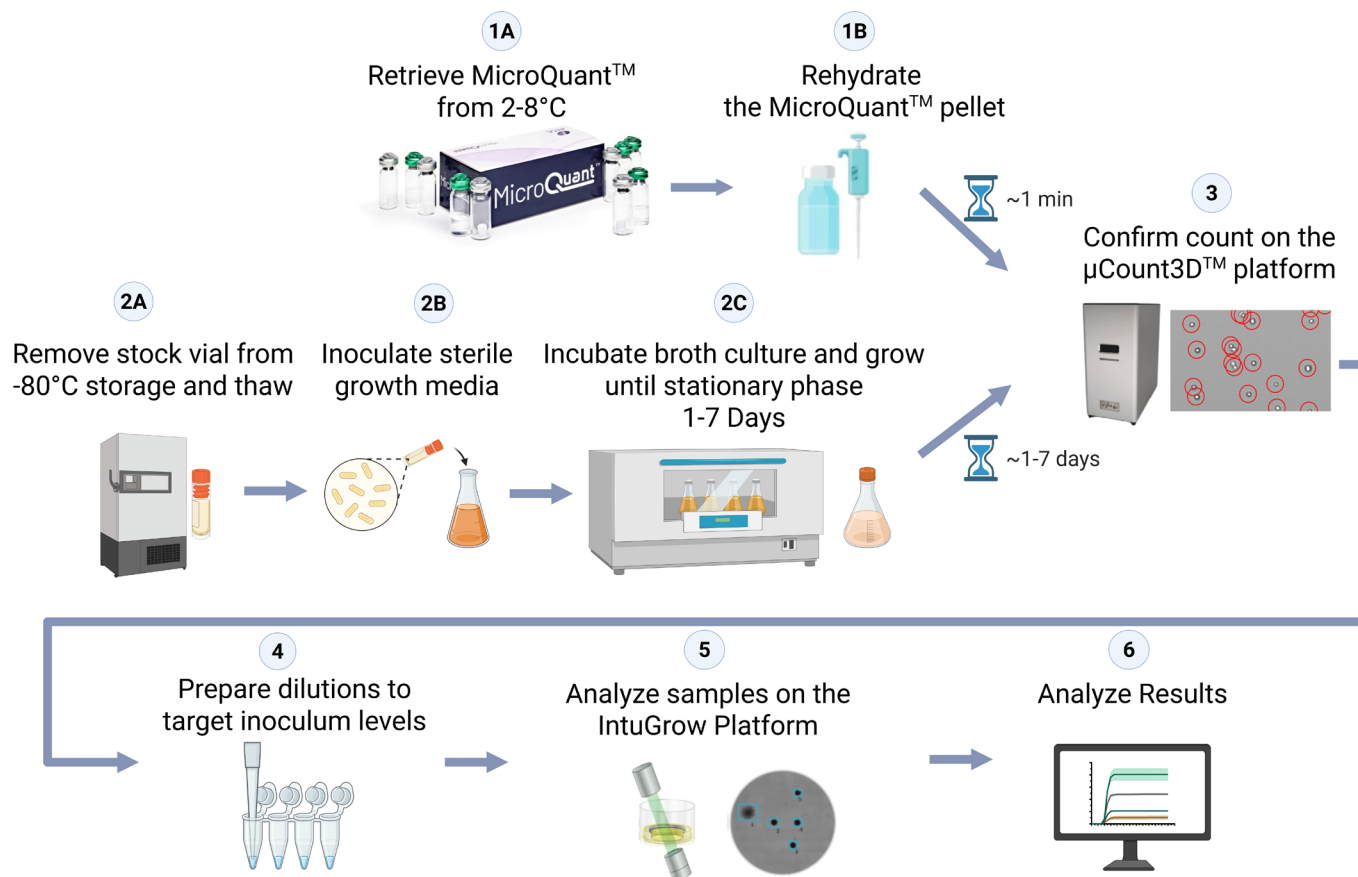


Figure 2: Comparison of inoculum preparation workflows – MicroQuant™ vs conventional culture for RMM testing. The schematic contrasts two approaches for generating microbial inocula prior to analysis. In the MicroQuant™ workflow, (1A) ready-to-use MicroQuant™ vials are retrieved from 2–8°C storage and (1B) rapidly rehydrated (~1 minute) to produce a quantified starting suspension. In the conventional workflow, (2A) microorganisms are recovered from frozen stock or culture plates, followed by (2B) subculturing and incubation to achieve sufficient growth, typically requiring 18–24 hours or up to several days depending on organism type. (2C) The resulting culture is then prepared for analysis, converging with the MicroQuant™ workflow. (3) Prepared suspensions are introduced into the μCount3D™ platform, after which (4) serial dilutions are prepared as required. (5) Samples are analyzed using the IntuGrow detection system, and (6) results are processed and reported. This comparison highlights the substantial reduction in preparation time and improved standardization achieved with MicroQuant™, minimizing variability associated with growth phase and operator-dependent handling in traditional workflows. Created with BioRender.com.

Inoculum Preparation and Enumeration with μCount3D™

All MicroQuant™ products were retrieved from 2–8°C storage and rehydrated according to their respective Instructions for Use. Following rehydration, the starting concentration of each MicroQuant™ suspension was confirmed using the μCount3D™ automated microbial cell counting system. The suspensions were then diluted to target concentrations in sterile, filtered saline buffer. Dilutions were enumerated in triplicate using μCassettes on the μCount3D™, and measured concentrations were used to calculate appropriate serial dilutions to achieve the desired target inoculum levels. All dilutions were prepared in accordance with pharmacopeial best practices. For the workflow comparison study, conventional inocula were prepared through recovery and expansion of microorganisms from stock cultures using standard microbiological procedures prior to dilution and analysis.

Growth Detection with IntuGrow

Prepared inoculum suspensions were analyzed using the IntuGrow platform, with each dilution tested in triplicate. For method comparability studies, samples were evaluated in parallel via the compendial plate-count method, using Tryptic Soy Agar (TSA) plates for bacterial species and Sabouraud Dextrose Agar (SDA) plates for fungal species. Parallel analysis enabled direct comparison of quantitative performance between methods. Incubation conditions were maintained in accordance with applicable pharmacopeial specifications.

Statistical Analysis

Linearity was assessed by linear regression of IntuGrow-derived results against compendial plate count results across a minimum of four concentration levels. Recovery was calculated as a percentage relative to the compendial method. Precision was evaluated by coefficient of variation (CV) across replicate measurements at target concentrations. All statistical analyses were performed in accordance with USP <1223> guidance on the validation of alternative microbiological methods.³

Results and Discussion

MicroQuant™ enabled near-unity linearity ($R^2 \geq 0.95$) between IntuGrow and compendial plate count results

Across all four organisms, IntuGrow-derived results demonstrated strong quantitative agreement with compendial plate count results. Linear regression analysis of IntuGrow values versus plate-count values yielded R^2 values ≥ 0.95 across the evaluated concentration ranges, exceeding the commonly applied pharmacopeial acceptance criterion (Figure 3). Further, slopes approximating unity indicate strong proportional agreement between methods (Figure 3).

The observed agreement between methods supports the use of MicroQuant™ as a standardized and quantitatively reliable inoculum for method comparability assessments. By providing a well-defined CFU input, MicroQuant™ minimizes variability introduced during inoculum preparation and enables more consistent assessment of linearity, recovery, and equivalence. These findings highlight the value of pre-quantified reference microorganisms in supporting robust and reproducible RMM validation workflows.

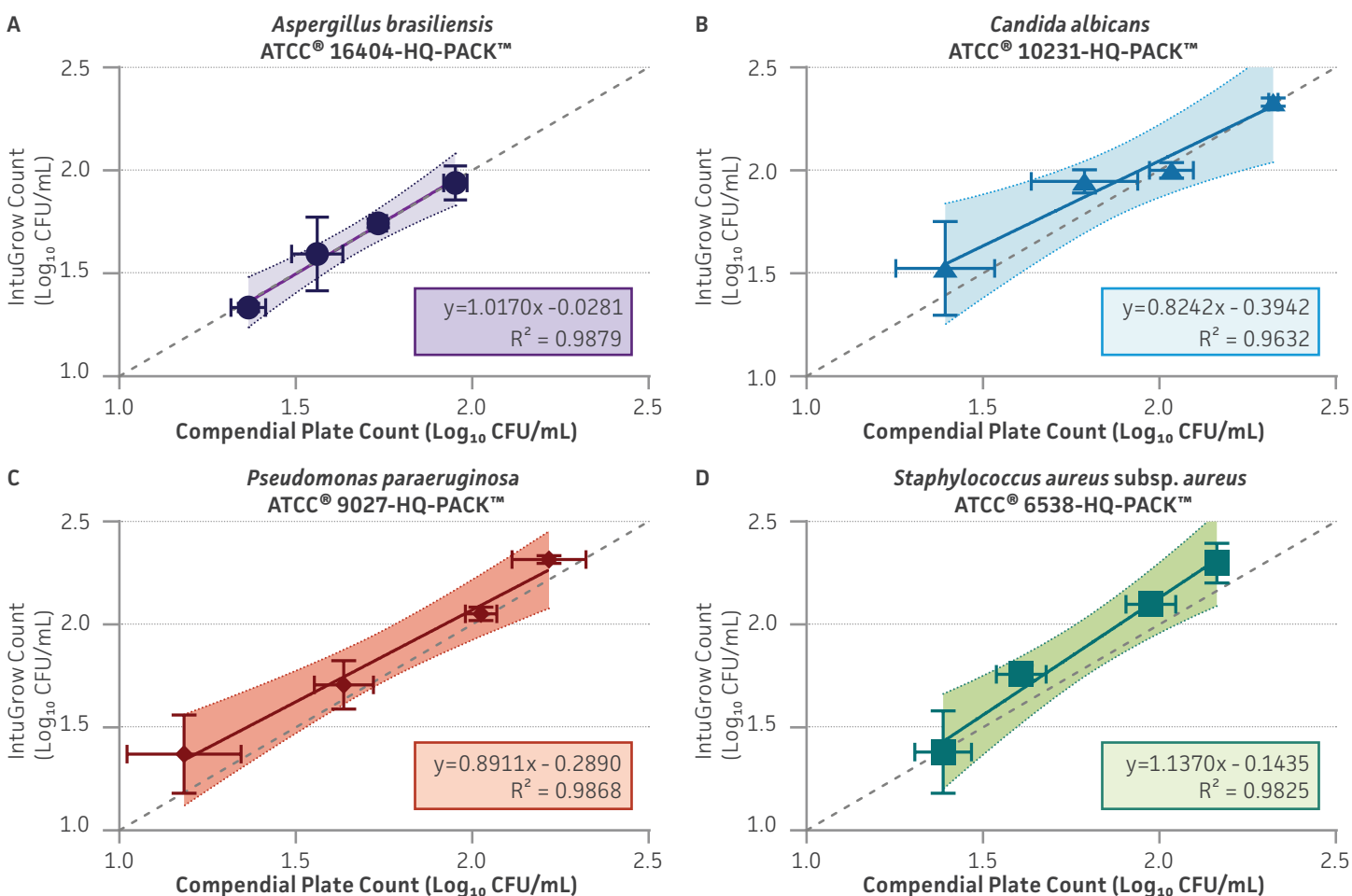


Figure 3: Linearity between IntuGrow-derived results and compendial plate count results using MicroQuant™ reference microorganisms. Linearity was evaluated for (A) *Aspergillus brasiliensis* (ATCC® 16404-HQ-PACK™, target concentrations = 12.5, 25, 50 and 100 CFU/mL), (B) *Candida albicans* (ATCC® 10231-HQ-PACK™, target concentrations = 25, 50, 100 and 200 CFU/mL), (C) *Pseudomonas paraeruginosa* (ATCC® 9027-HQ-PACK™, target concentrations = 25, 50, 100 and 200 CFU/mL), and (D) *Staphylococcus aureus* subsp. *aureus* (ATCC® 6538-HQ-PACK™, target concentrations = 25, 50, 100 and 200 CFU/mL). Data plotted at Log10 scale for the regression analysis. Each data point represents the average of three independent replicate measurements. The dashed line indicates the line of identity. The acceptance criterion was defined as $R^2 \geq 0.95$.

MicroQuant™ delivered method-equivalent precision with no significant CV% difference between IntuGrow and compendial plate count methods

Coefficient of variance (CV%) analysis across five serial dilution levels demonstrated robust repeatability and intermediate precision, consistent with USP <1223> expectations. Mean CV% values for the IntuGrow platform and compendial plate count methods were not significantly different across the evaluated organisms (one-way ANOVA showed no statistical difference between methods), with variability remaining within acceptable limits as defined by the validation criteria (Figure 4). Together, these findings indicate consistent control of inoculum input across dilution levels and measurement platforms.

Reliable control of inoculum variability is critical for RMM validation, as inconsistent microbial inputs can compromise method performance and lead to repeat testing. The low variability observed across dilution levels highlights the value of MicroQuant™ as a standardized, pre-quantified inoculum source. By eliminating culture expansion and reducing manual quantification steps, MicroQuant™ minimizes common sources of variability associated with traditional inoculum preparation workflows, supporting more reproducible validation studies and streamlined implementation of alternative microbiological methods.

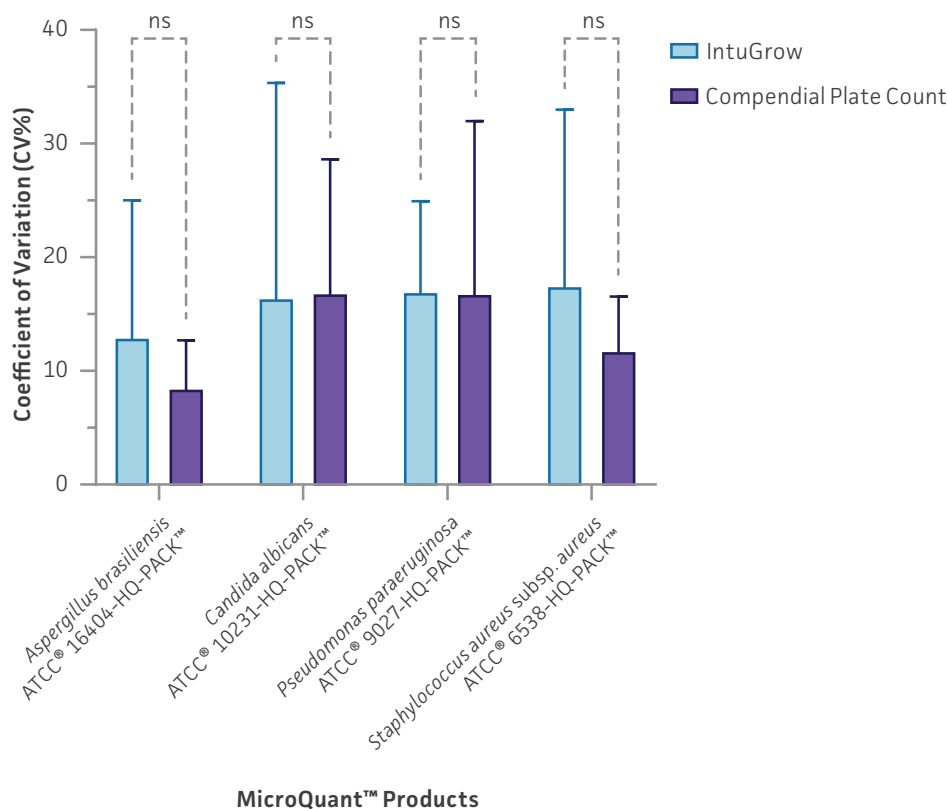


Figure 4: Precision assessment of the IntuGrow system and compensial plate count method across MicroQuant™ reference microorganisms. The coefficient of variation (CV%) was calculated at five target dilution levels with three independent replicates per dilution (N = 3 replicates x 5 dilutions = 15 per organism). Bar heights represent the mean CV% across the dilution levels, and error bars indicate the standard deviation of dilution-specific CV% values. Dashed brackets denote statistical comparisons between methods, evaluated by one-way ANOVA followed by Tukey's Post-hoc analysis (ns = no significant difference).

MicroQuant™ shortened time-to-result with IntuGrow versus overnight culture while maintaining recovery (92.5-112.7%) and tighter precision (CV% ≤ 20%)

MicroQuant™ streamlined inoculum preparation and reduced overall workflow timelines compared with conventional culture-based approaches. Consistent with RMM terminology, time-to-detection (TTD) is the elapsed time at which the IntuGrow growth signal exceeded predefined detection threshold. Time-to-result (TTR) is defined as the time required to generate a reportable analytical result following sample inoculation. TTD for MicroQuant™ inocula ranged from 4–6 h for bacterial species and 6–8 h for fungal species, compared with 4–8 h and 8–12 h, respectively, for overnight culture preparations. Similar trends were observed for TTR, with bacterial results obtained within 6–13 h for MicroQuant™ versus 12–20 h for overnight culture, while fungal TTR remained comparable at 14–24 h (Figure 5). These findings demonstrate that MicroQuant™ can accelerate RMM workflows by eliminating culture expansion and reducing the time required to generate standardized test inocula without compromising analytical performance.

Quantitative performance of the IntuGrow platform using MicroQuant™ inocula met or exceeded compensial expectations for recovery. The recovery values ranged from 92.5% to 112.7%, well within the accepted 50–200% range defined by USP <61> and related pharmacopeial guidance. This high level of recovery demonstrates that the pre-quantified MicroQuant™ format provides a reliable, well-defined input material for RMM validation and method comparability studies.

Precision analysis further highlighted the benefits of standardized inoculum preparation. CV% values for MicroQuant™ remained ≤20%, substantially lower than the ≤35% acceptance threshold and consistently below variability observed with overnight cultures (≤35%). This enhanced precision reflects tighter control of inoculum input facilitated by the single-use, pre-quantified format of MicroQuant™, which reduces variability associated with culture growth, manual enumeration, and operator-depending handling. Together, these results indicate that MicroQuant™ supports more reproducible validation studies while reducing the variability that can contribute to repeat testing and extended validation times.

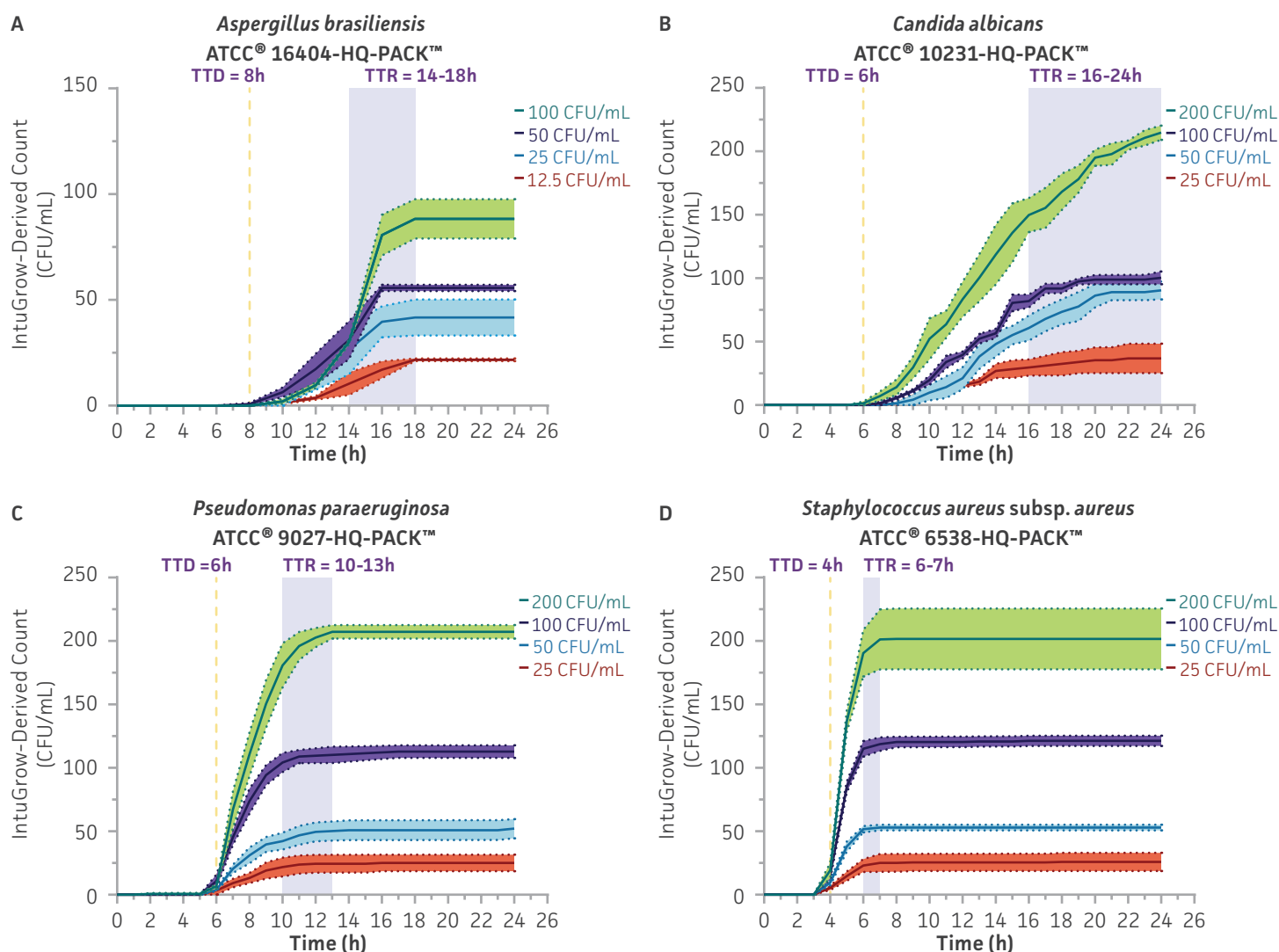


Figure 5: Representative IntuGrow growth curves generated using MicroQuant™ reference materials. Growth kinetics were evaluated for (A) *Aspergillus brasiliensis* (ATCC® 16404-HQ-PACK™), (B) *Candida albicans* (ATCC® 10231-HQ-PACK™), (C) *Pseudomonas paraeruginosa* (ATCC® 9027-HQ-PACK™), and (D) *Staphylococcus aureus* subsp. *aureus* (ATCC® 6538-HQ-PACK™). Each curve represents the mean IntuGrow-derived count obtained at the indicated target inoculum concentration (CFU/mL). Shaded bands surrounding each curve indicate the standard deviation of replicate measurements. The dashed vertical line denotes the time-to-detection (TTD), and the shaded blue region represents the time-to-result (TTR) window.

Table 2: Comparison of IntuGrow platform performance using MicroQuant™ reference microorganisms and traditional overnight culture preparation.

Parameter	MicroQuant™	Overnight culture	Acceptance Criteria (Compendial expectation)
Time to Detection (TTD)	4-6 h (bacterial)	4-8 h (bacterial)	Organism-dependent
	6-8 h (fungal)	8-12 h (fungal)	
Time to Result (TTR)	6-13 h (bacterial)	12-20 h (bacterial)	Organism-dependent
	14-24 h (fungal)	14-24 h (fungal)	
% Recovery	92.5%-112.7%	50-200%	50-200%
Coefficient of Variation (CV%)	≤20%	≤35%	≤35%

Conclusion

The successful implementation of rapid microbiological methods (RMMs) depends on the ability to generate reproducible, well-characterized microbial inocula for method validation. In this study, MicroQuant™ quantitative reference microorganisms provided a standardized and reliable inoculum source for the validation of RMM workflows. When used in combination with the µCount3D™ automated microbial cell counting system and the IntuGrow rapid growth-based detection platform, MicroQuant™ enabled strong agreement with compen-

dial plate-count methods, with linearity ($R^2 \geq 0.95$), recovery, and precision meeting established pharmacopeial acceptance limits.

Beyond analytical performance, MicroQuant™ simplified inoculum preparation by eliminating culture expansion and reducing variability associated with growth phase, manual enumeration, and operator-dependent handling. Compared with conventional inoculum workflows, the use of MicroQuant™ improved workflow efficiency while maintaining robust quantitative performance, supporting faster generation of validation-ready data.

Collectively, these findings demonstrate that MicroQuant™ can serve as a foundational tool for standardizing RMM validation workflows. By providing a ready-to-use, pre-quantitative microbial reference material, MicroQuant™ helps reduce a key source of variability in method validation, enabling laboratories to implement alternative microbiological methods with greater confidence, reproducibility, and operational efficiency. As the pharmaceutical industry continues to adopt data-rich microbial testing strategies, standardized reference materials like MicroQuant™ can play an important role in accelerating validation while maintaining compliance with USP <1223> and EP-5.1.6 requirements.

Why MicroQuant™ for RMM Validation?

MicroQuant™ addresses the most impactful sources of variability and inefficiency in RMM validation workflows:

- Provided at a defined CFU range (10^2 – 10^3 CFU/vial for low-CFU format and 10^7 – 10^8 CFU/vial for high-CFU format), eliminating the need for upfront quantification and enabling confident inoculum control from the first step.
- Requires no overnight growth, culture media, or incubation equipment at the preparation stage, reducing total culture preparation time to under 5 minutes and minimizing operator-dependent variability.
- Derived from passage-zero, authenticated ATCC reference strains cited in USP, ensuring full regulatory alignment for compendial suitability testing.
- Produced under ATCC's ISO 17034–accredited biomanufacturing facility and evaluated in ISO/IEC 17025 quality system, with lot-to-lot consistency verified and traceable to the reference biomaterials.
- Stable at 2–8°C for up to 24 months (see product-specific expiry), eliminating the need for –20°C freezer infrastructure or thaw-cycle management.
- Compatible with growth-based detection platforms (e.g., IntuGrow) and automated microbial cell counting system (e.g., µCount3D™), supporting a range of RMM validation strategies.

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