

Miniaturized Microplate Readers for High-Throughput Growth Analysis of Anaerobic Bacteria

WHO THIS WHITE PAPER IS FOR

- ▶ Research Scientist ▶ Industrial Microbiologists
- ▶ Lab Manager ▶ Environmental Scientist
- ▶ Principal Investigator ▶ Assistant/Associate Professor
- ▶ Microbiologist ▶ Postdoctoral Researcher

FIELDS OF IMPACT

Academic and Research Institutions

- ▶ Universities
- ▶ Government-funded labs (NIH, CDC, EPA, USDA - ARS)
- ▶ Independent research organizations

Biotechnology and Pharmaceuticals

- ▶ Probiotic development
- ▶ Antimicrobial research
- ▶ Gastrointestinal diseases

Environmental Science

- ▶ Bioreactors and waste treatment
- ▶ Soil microbiomes, carbon sequestration and methanogenesis

Agriculture and Animal Health

- ▶ Soil health labs
- ▶ Animal nutrition companies



The following white paper is based on the 2024 publication Scale-up of industrial microbial processes: Snoeyenbos-West OLO, Guerrero CR, Valencia M, Carini P.2024. Cultivating efficiency: high-throughput growth analysis of anaerobic bacteria in compact microplate readers. *Microbiology Spectrum* 12:e03650-23.

<https://doi.org/10.1128/spectrum.03650-23>.



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The Stratus microplate reader discussed in this paper has now been commercially replaced by the next generation, advanced version, the **Alto**.

Alto® Features • • • • • • • • •



Compact and Portable

Design: Fits tight lab spaces, including anaerobic chambers.



Compatibility with

Multiple Plate Types: Supports 6, 12, and 96-well plates.



Flexible Measurement

Modes: Continuous real-time or endpoint measurements.



► Introduction

Given the more complex environment and conditions required to study anaerobic bacteria, researchers are understandably wary of adopting new tools and techniques. They continue to use tried and tested tools since they have been reliable, even if those tools are cumbersome and slower than their newer counterparts. However, it can also be a source of frustration and a pain point for researchers because the process isn't evolving toward improvement.

Miniaturized microplate readers are a newer tool that has been studied extensively, and based on comparison with traditional standard plate readers, they have repeatedly proven to be more efficient without sacrificing the quality of the results. There is increasing interest in miniaturized microplate readers to analyze anaerobic bacteria as their results have been just as reliable as those of the traditional methods.

Anaerobic bacteria present a unique challenge because of the difficulty in establishing the appropriate conditions. There are numerous known issues with the current tools used to monitor and analyze the phenotypic behavior of anaerobic bacteria. To alleviate some of the most significant issues with traditional tools, the authors of a recent study compared the performance of Stratus, Cerillo's small-footprint microplate reader, against a larger, more widely used microplate reader.

Cerillo's small-footprint microplate readers are designed to assuage concerns about the reliability and accuracy of this more efficient method, providing a high-throughput growth analysis in less time with comparable results. Further, when up to 5 Cerillo plate readers are paired with Canopy, users can control, monitor, and record from each device simultaneously thus ensuring fewer variables, such as denigration of a sample or improper cleaning of



an instrument between uses. This means that the resulting data is more accurate as it is available for review and interpretation sooner. As a result, action can be taken more quickly if it is necessary.

The study found that the tool provides a solution to several of the pain points of more commonly used tools, especially their size. Cerillo's microplate readers are compact and portable, allowing researchers and labs to use the device where it is needed instead of having to create the environment around it. Designed for wireless connectivity, users do not need cables to connect with a data acquisition computer. The tool has a high environmental tolerance, so it can be used in low oxygen, high humidity, and within a wide range of temperatures. Because of the device's portability, it allows researchers and technicians to use Cerillo's microplate readers in the field.

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Another significant issue with complex microplate readers is that they are cost-prohibitive, both at initial purchase and with necessary service agreements throughout the product's lifespan. These traditional readers are often a shared resource in labs and within organizations, so they may not be available when needed. Given their size, larger readers typically cannot be moved, so the analyses must be done where the tools are located. When this is a shared space, there is also a risk of unknown cross-contamination.

Finally, with its small footprint, Cerillo's new microplate reader (the Alto) has two measurement modes (continuous real-time or endpoint

measurements) that are compatible with multiple plate types. This allows users to work with the available tools instead of requiring a large complex device to begin interpreting the results.

While it is understandable that adapting to new tools and devices is a slow process, Cerillo's compact microplate reader has demonstrated that it is as effective as comparable, larger microplate readers. At the same time, it provides results much more quickly, with fewer limitations on space and environmental conditions. Ultimately, implementing a plan to start using Cerillo's microplate reader, at least for some of the work, can provide relief from some of the problems caused by the larger tools.





► Methodology

To test the results of Cerillo's small footprint microplate reader, the researchers ran an identical analysis using the BioTek Synergy HTX microplate reader. Both were used to study *Escherichia coli* DSM 28618.

The researchers paired the Stratus with a Grant Instrument Microplate Shaker PMS-1000i so that it would fit in the COY Model 2000 Force Air Incubator for Vinyl Chambers. The growth was tracked using custom scripts and Growthcurver software.

The BioTek Synergy HTX used a traditional configuration and output setup. It is a traditional microplate reader for monitoring microbial growth, and probably a tool that many people have used at some point during their careers.

Prior to starting the comparison, researchers developed a unique process flow for Cerillo's microplate reader. Users defined the time points, and the small-footprint microplate reader captured OD600 data and temperature measurements based on the user input. The data was then stored either as a .csv file or a .txt file.

Once the parameters and workflows were established, the researchers worked with aerobic cultures. After the first round of tests, they ran a similar test designed for anaerobic cultures. While the study included both aerobic and anaerobic bacteria, the aerobic results were used as a baseline for understanding the anaerobic results. Establishing the right kind of environment for anaerobic cultures is of greater concern because it is harder to create and maintain it.

► Key Findings

The study started with establishing a baseline understanding of how comparable Cerillo's microplate reader is to the BioTek microplate reader. That meant starting with aerobic cultures. Once the aerobic culture results were obtained, the researchers were better able to test the anaerobic conditions.

Aerobic cultures were allowed to grow overnight. These were added to two 96-well microtiter plates with lysogeny broth media. They were then added

to 64 wells. 32 wells were not inoculated as they acted as the control plates.

The study's authors compared the efficacy of the small-footprint microplate reader to accurately represent the growth curve data against the growth rate results of BioTek's microplate reader. Both the growth rates and carrying capacities of the two devices were found to be comparable, with very little differences between them.

	Growth Rate	Mean Carrying Capacity (k)
Small-footprint microplate reader	$1.24 + 0.06 \text{ h}^{-1}; n = 64$	$0.51 + 0.02; n = 64$
BioTek	$1.28 + 0.02 \text{ h}^{-1}; n = 64$	$0.47 + 0.01; n = 64$

However, the area under the curve (AUC) was significantly different.

	AUC
Small-footprint microplate reader	$10.2 + 0.36 \text{ h}^{-1}; n = 64$
BioTek	$7.65 + 0.21 \text{ h}^{-1}; n = 64$

The data from the Stratus were bimodally distributed for the three growth parameters. This difference is theorized to be a result of differences in the shaking process, which could have affected the oxygenation.

With confirmation in the aerobic conditions, the same comparison was run under anaerobic conditions. The focus was on quantifying the AUC of *E. coli* DSM 28618 and *Clostridium bolteae* DSM 29485. Both types of cultures were from freezer stocks, and 200 μL of the cultures were anaerobically injected into the plates. Then, eight

different doses (0, 0.4, 1.3, 3.8, 38.5, 116, 335, or 1,039) of arsenite were added.

The mean AUC was significantly different across the applied concentrations of arsenite. For lower doses (3.8 and less), the AUC was reduced. Doses over 335 μM significantly inhibited growth. The results aligned with expectations.

The authors published their full study for those who want to take a closer look at the data and how the full testing was run.

► Conclusion

It is already difficult to conduct tests in anaerobic conditions, so the study focused on ensuring that Cerillo's small footprint microplate reader was able to provide the same accuracy as a larger microplate reader. The authors found that it was not only comparable in terms of accuracy, but it was more effective and efficient.

The authors found Cerillo's miniaturized microplate readers to be a tool that has a high-throughput cultivation and growth analysis pipeline making it more efficient, compact, and cost-effective. During simultaneous tests on Cerillo and BioTek microplate readers, researchers were able to complete the testing on the Cerillo microplate reader more easily as it was compact, allowing it to fit more easily into a wider range of spaces. It performed consistently across the different tests, providing comparable results more quickly and with less extra work (such as preparing a space or managing a lot more hardware). As there was less manual intervention, the testing was simpler and had a lower risk of human error in the final results.

While the authors didn't take cost into account for the testing, Cerillo's products are priced to be more affordable, both at initial purchase and over the instrument's lifespan. Working with larger models often requires additional planning so that testing doesn't overlap with other projects or researchers who also need to use the device. It is also incredibly user-friendly because it is made to be versatile. The set up process is simple, and the software is comparable to that of other devices. The fact that it can be taken into the field, an option not available with traditional tools, provides an advantage that can make this device an essential part of testing and research going forward.





Alto Microplate Reader

Cerillo's microplate reader, Alto, is a compact, efficient, and wireless standalone solution for researchers studying anaerobic microbes.

Combining portability with real-time data monitoring, analytical software and seamless workflow integration, Alto empowers scientists to achieve breakthrough results in diverse settings, from lab controlled environments to field applications. By reducing infrastructure requirements and enabling smarter, more collaborative research, Cerillo's products enhance productivity, lower operational costs, and position labs for future innovation, making it an indispensable tool for advancing anaerobic science.



Offers flexible measurement modes for a wide range of experiments



Ideal size for compact spaces (e.g., shared benches, incubators, chambers)



Operates in challenging environments where others can't



User-friendly software for quick data analysis and meaningful results

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