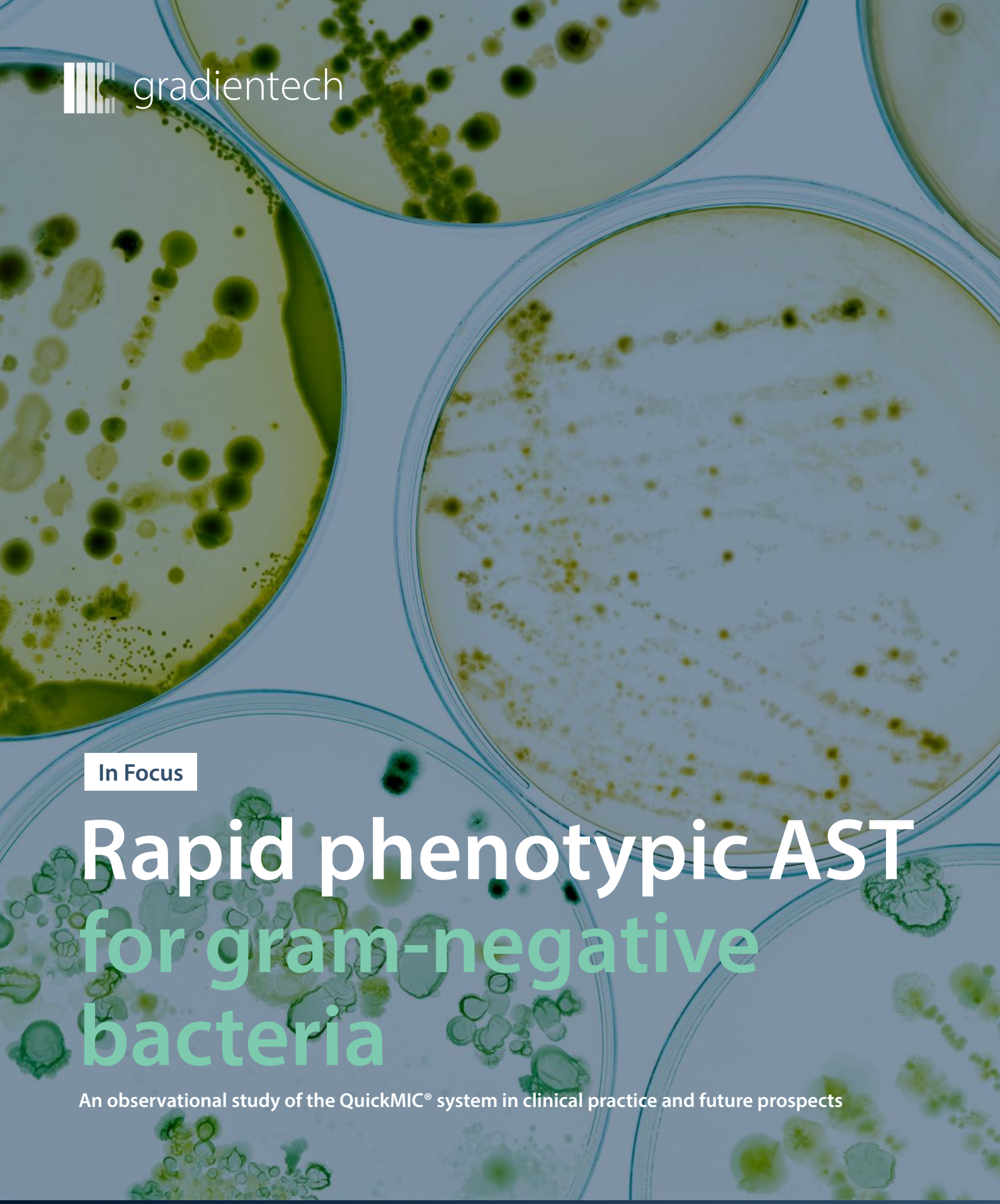

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In Focus

Rapid phenotypic AST for gram-negative bacteria

An observational study of the QuickMIC® system in clinical practice and future prospects

Authors & Affiliations

This in focus paper is compiled by Gradientech presenting data from the article: García-Rivera C, Ricart-Silvestre A, Parra Grande M, Ventero MP, Tyshkovska-Germak I, Sánchez-Bautista A, Merino E, Rodríguez JC. 2024. **Evaluation of the QuickMIC system in the rapid diagnosis of gram-negative bacilli bacteremia**. Microbiol. Spectr. 12:10. The study was funded in part by Gradientech on a cost-per-sample basis. Reagents, instruments, and consumables were provided by Gradientech.

The challenges of gram-negative bacteria and sepsis

The emergence of antimicrobial resistance (AMR) proposes a challenge for empirical antibiotic therapy and shows the need for faster diagnostics to guide treatments. The request for rapid antibiotic susceptibility testing of gram-negative bacilli is especially needed in cases of sepsis. An overall mortality rate of 11 % has been reported, with inadequate treatment being a significant risk factor.[1,2] When sepsis is caused by multidrug-resistant microorganisms, mortality rates can reach up to 50 %.[3] In addition to death and disability, AMR has significant economic costs. The World Bank estimates that AMR could result in US$ 1 trillion in additional healthcare costs by 2050.[4] Rapid confirmation or adjustment of antibiotic treatment could contribute to a reduction in healthcare costs.

This in focus paper summarises the highlights from an observational study and explores how the QuickMIC® system could contribute to more efficient sepsis patient management.

Study highlights

The QuickMIC® system can facilitate **early confirmation or adjustment** of treatment → The concordance between the QuickMIC® system and reference methods was **99.02%** → The average analysis time for the QuickMIC® system ranged **from 2-4h**

Study design

This observational study was designed to evaluate the performance of the QuickMIC® system (Gradientech, Uppsala, Sweden) in clinical practice at the Dr. Balmis University General Hospital (Alicante, Spain) in a setting with a high incidence of multidrug-resistant bacteria.

- ✓ **Inclusion criteria** Monomicrobial bacteremia caused by gram-negative bacilli, including species which the QuickMIC® system is validated for.
- ✓ **Exclusion criteria** Bacteremia caused by other pathogens, polymicrobial or non-validated bacterial species.

Diagnostic workflow for positive blood cultures

The bacterial species were identified using MALDI-TOF (Bruker). Real-time PCR (Gene-Xpert, Cepheid) and immunochromatography (Biotech) systems were used for the rapid detection of the main resistance mechanisms, extended-spectrum beta-lactamases and carbapenemases (Figure 1). Antimicrobial susceptibility testing (AST) was performed using the MicroScan WalkAway plus system (Beckman Coulter). Borderline or anomalous susceptibility results, especially for colistin susceptibility, were confirmed using the E-test (bioMérieux) or a microdilution system. The QuickMIC® ultra-rapid AST system was implemented into the routine workflow and was run after MALDI-TOF identification.

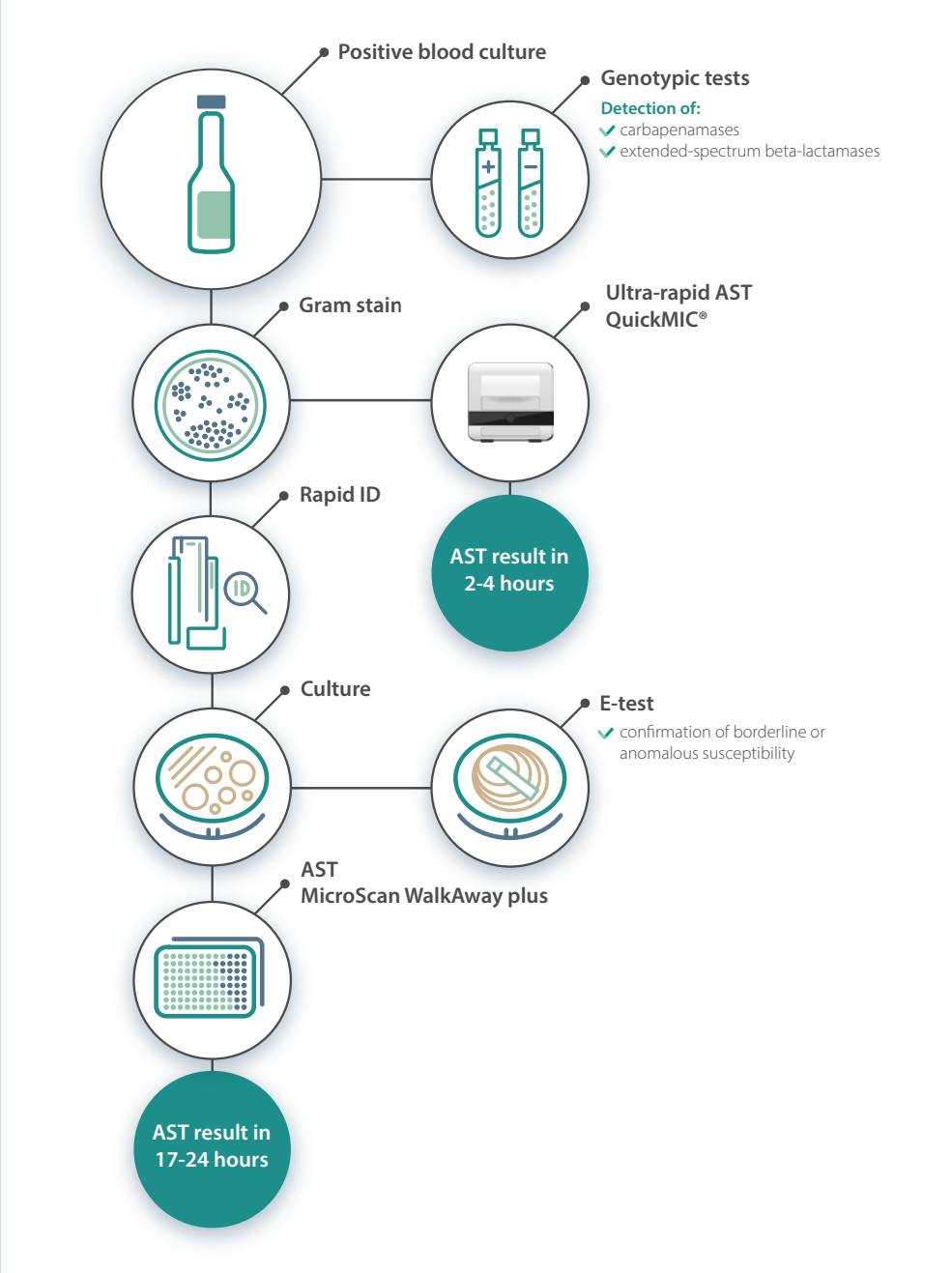

Figure 1. Workflow for the routine protocol for processing positive blood cultures for gram-negative bacteria and the alternative workflow for ultra-rapid AST using the QuickMIC® system.

Results

QuickMIC delivers higher sensitivity and specificity with a shorter turnaround time

68

Positive blood cultures
Bacterial species included *E. coli, K. pneumoniae, P. aeruginosa, Proteus* spp. and *S. marcescens.*

816

Drug-bug combinations
Each sample was run against a panel containing 12 antibiotics.

>99 %

Concordance rate
99.02% concordance rate between the QuickMIC® system and the MicroScan WalkAway plus system.

3 hours

Average analysis time
for the QuickMIC® system compared to 17-24 hours for the MicroScan WalkAway plus system

During the study, 86 positive blood flasks were obtained; of these, 18 were excluded. The species distribution was *E. coli* (n = 41, 60.3%), *Klebsiella* spp. (n = 14, 20.5%), *Enterobacter* spp. (n = 5, 7.4%), *P. aeruginosa* (n = 4, 5.9%), *Citrobacter* spp. (n = 2, 2.9%), *Proteus* spp. (n = 1, 1.5%), and *Serratia marcescens* (n = 1, 1.5%). Out of the 816 drug-bug combinations tested, eight discrepancies were found, which resulted in a concordance rate of 99.02%. The turnaround time was significantly reduced from 41-48 hours to 4-6 hours using the QuickMIC® system (Table 1).

> This new system is especially relevant for analysing antibiotic susceptibility in gram-negative bacilli against first-line medications, where rapid protein or gene-based tests are lacking."

The QuickMIC® system

- An ultra-rapid phenotypic AST system developed by Gradientech
- Samples can be run directly from positive blood cultures
- The QuickMIC® GN panel contains 12 antibiotics tailored towards common sepsis-causing bacteria
- Validated for *E. coli*, *K. pneumoniae* complex, *K. oxytoca*, *K. aerogenes* and *K. variicola*, *P. aeruginosa*, *A. baumannii-calcoaceticus* complex, *Enterobacter cloacae* complex, *P. mirabilis*, *P. vulgaris*, *C. koseri*, *C. freundii* and *S. marcescens*.

The QuickMIC® system and its gram-negative panel are CE marked. The QuickMIC® system is not FDA 510(k)-cleared and not available in the U.S.

	QuickMIC®	MicroScan WalkAway plus
Analysis time	2-4 h	17- 24 h
Turnaround time*	4-6 h	41-48 h

Table 1: The routine AST system for analysing positive blood flasks takes 17-24 hours, while QuickMIC® shortens this time to 2-4 hours.
*Turnaround time: the total time from positive blood culture to AST result at Dr. Balmis University General Hospital laboratory, was shortened when using QuickMIC® from 41-48 hours to 4-6 hours.

"Given the existing limitations of currently available rapidAST methods, the clinical utility of QuickMIC® is particularly relevant in the management of *P. aeruginosa* infections and AmpC-producing Enterobacterales. The use of such rapid methods can help diversify antibiotic use and reduce carbapenem consumption."

Rapid MIC values could facilitate personalised treatment options

The QuickMIC® system demonstrates exceptional performance in ultra-rapid antibiotic susceptibility testing, surpassing traditional methods in both sensitivity and specificity while significantly reducing turnaround time. The study revealed that QuickMIC® efficiently determines the effect of 12 antibiotics against the primary gram-negative bacilli associated with bacteremia, offering critical information for the timely adjustment for escalation or de-escalation of antibiotic therapy. By providing precise and early minimum inhibitory concentration (MIC) values, the system enables targeted therapy for patients and contributes to the fight against antibiotic resistance. The study suggests that QuickMIC® is particularly advantageous in managing *P. aeruginosa* infections, where existing rapid diagnostic methods face significant limitations due to their antibiotic coverage.

Clinical utility of rapid AST systems — future prospects

The need for a rapid AST result is not only crucial for escalation or de-escalation of treatment, rapid AST is also needed for "difficult-to-treat" pathogens as they are not usually susceptible to first-line treatments. An ultra-rapid AST result would not only confirm that a patient has received the right treatment but also make sure that the initial therapy is adjusted within a short period of time. In addition, an ultra-rapid result would also facilitate appropriate measures to control the spread of these strains in the hospital environment.[5] Other advantages include positive impact on morbidity and length of hospital stay, which in turn leads to reduced hospital costs.[5,6] While phenotypic ultra-rapid AST systems offer clear clinical advantages, it is important to consider how these methods compare to genotypic approaches in detecting antimicrobial resistance.

Genotypic vs. phenotypic diagnostic systems: navigating the future of rapid AST

A phenotypic AST system capable of providing ultra-rapid MIC values presents a robust alternative to protein- or gene-based diagnostic solutions (Table 2). Phenotypic methods directly measure bacterial behaviour in response to antibiotics. This is particularly valuable for species like *P. aeruginosa* that exhibit high variability due to resistance mechanisms involving multiple genetic and environmental factors. These systems capture the complexity of bacterial resistance in real time, offering clinicians actionable results to tailor treatments early in the infection process. In contrast, protein- and gene-based diagnostics detect specific genetic markers or resistance genes, which can offer earlier detection but are often limited by the complexity of resistance mechanisms. Genotypic tests can also miss novel or rare resistance mechanisms that have not been fully characterised.[7]

Genotypic resistance detection	Phenotypic susceptibility testing
+ Viable bacterial growth not required for detection of genes	+ Less dependent on the resistance mechanisms present
+ Detection of genes in polymicrobial samples possible	+ May detect resistant bacteria with novel and unknown resistance mechanisms
+ System validation for each bacterial species not required, only the detection of each gene	+ The antibiotic effect is directly measured
+ Usually rapid turnaround, directly from sample	+ The result can be used for estimating clinical benefit effect of the drug through breakpoints, and guide therapy
− The result cannot readily be used for estimat-ing clinical effect of the drug, with exceptions	− Viable bacterial growth required for detection
− Presence of gene does not necessarily correlate with actual resistance	− Analysis of polymicrobial samples difficult, requires a method for separation
− Resistance is usually multifactorial involving multiple and genes and cannot be quantified	− System validation for each bacterial species required, performance can depend on species
− A library of resistance genes is needed, unknown and new mechanisms may go undetected	− Usually slow turnaround, sample preparation typically needed

Table 2: Advantages and disadvantages for genotypic resistance detection and phenotypic susceptibility testing.

The difficulty of translating genotype into phenotype remains a critical limitation of molecular diagnostics, especially in cases where bacterial resistance mechanisms are not yet fully understood. While protein- or gene-based diagnostics are effective for detecting known resistance markers, they sometimes fail to account for the antibiotic effect, leading to suboptimal treatment decisions for infections associated with complex resistance mechanisms.

Concluding remarks

This study contributes to the ongoing debate within the field of microbiology: *Can we ever fully catch up with bacterial evolution and reliably link genotype to phenotype?* As bacteria continue to evolve and generate novel resistance variants, the sheer number of genetic combinations influencing resistance approaches infinity, posing a challenge for molecular diagnostic tools. Phenotypic systems address this challenge by directly observing the in vitro antibiotic effect, making them indispensable in managing infections where resistance mechanisms are complex or not fully understood. Recent advances in machine learning and bioinformatics hold promise for improving the predictive power of genotype-based diagnostics.[6] Phenotypic ultra-rapid AST systems like QuickMIC®, offer a comprehensive and more reliable solution in clinical settings where timely and accurate information is critical.

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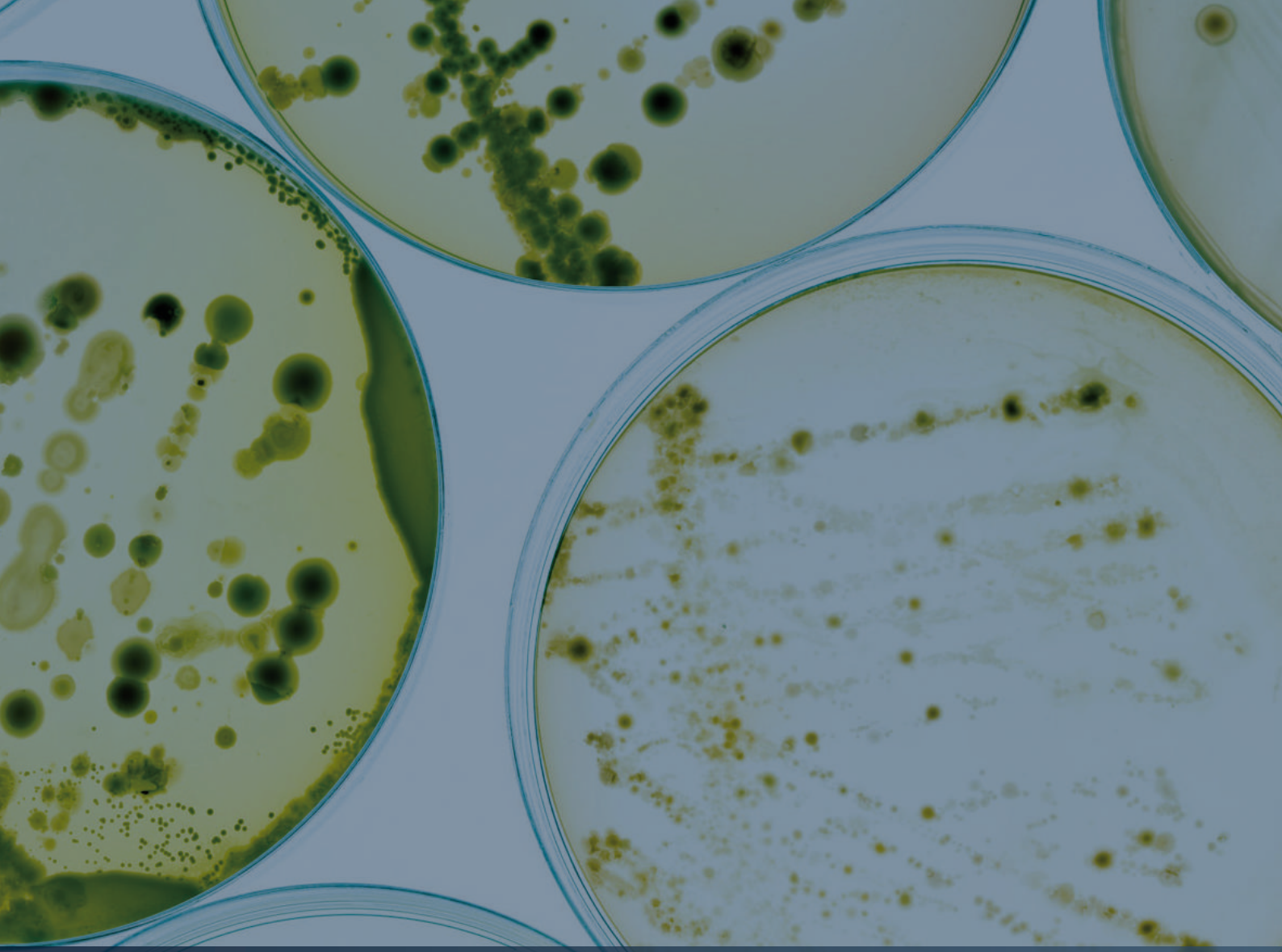

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