

Mini Review

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Laboratory Validation of EUCAST Rapid Antimicrobial Susceptibility Testing (RAST) and Its Clinical Impact on Sepsis Management

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Abstract

In sepsis, the most decisive modifiable factor influencing survival is the early administration of appropriate antimicrobial therapy. The turnaround time to obtain the conventional antimicrobial susceptibility testing results is 16–24 hours post-blood culture-positive results, which can delay targeted therapy administration and prolong empirical broad-spectrum antibiotic usage. The rapid antimicrobial susceptibility testing (RAST) method proposed by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) enables disk diffusion-based susceptibility interpretation directly from positive blood cultures within 4–8 hours. However, the clinical utility of RAST is dependent on rigorous laboratory validation to ensure acceptable analytical performance and minimize categorical errors. This mini review summarizes recent evidence on the EUCAST RAST methodology, validation requirements, analytical performance metrics, implementation strategies, and clinical impact in bloodstream infections and sepsis. The importance of local validation aligning with EUCAST quality control recommendations and the role of RAST in strengthening antimicrobial stewardship programs and supporting global antimicrobial resistance containment efforts have been discussed.

Introduction

Sepsis, a potentially fatal syndrome, is characterized by organ dysfunction resulting from a dysregulated host response to infection. Delayed initiation of effective antimicrobial therapy increases mortality risk in patients with sepsis, warranting the need for rapid and early intervention. In bloodstream infection (BSI) management, conventional antimicrobial susceptibility testing (AST) workflows typically involve overnight incubation after blood culture yields positive results, delaying therapeutic optimization^{1,2}.

Rapid diagnostic technologies aim to reduce the turnaround time of AST. Molecular platforms enable accelerated pathogen identification. However, phenotypic susceptibility data are essential for guiding definitive therapy. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) introduced the rapid antimicrobial susceptibility testing (RAST) method to provide standardized early disk diffusion-based results directly from positive blood culture bottles^{3,4}. Several multicenter studies and real-world implementation analyses have evaluated the analytical reliability and clinical utility of RAST⁵⁻¹⁴. However, rapid reporting should not compromise diagnostic accuracy. Thus, laboratory-specific validation must be performed to ensure patient safety and maintain performance within acceptable categorical error thresholds. This review examined the methodological framework of EUCAST RAST, the necessity of its local validation, and its clinical implications in sepsis management.

EUCAST RAST: Methodological Framework

In EUCAST RAST, the positive blood culture broth is directly inoculated onto Mueller-Hinton agar. Subsequently, the samples are subjected to standard disk diffusion testing. The zone diameter is measured at 4, 6, and/or 8 hours (with optional 16–20-hour readings where applicable) using species-specific and time-specific breakpoints defined by EUCAST^{3,4}. The key features of this RAST include time-dependent zone diameter breakpoints, areas of technical uncertainty (ATU), defined quality control (QC) ranges, and standardized interpretation tables. The RAST method is validated primarily for common BSI pathogens, including *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Salmonella* spp., *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Enterococcus* spp.^{3,5}. Recent updates have further refined resistance detection guidance and QC parameters, reinforcing the need for strict adherence to EUCAST methodology^{3,4}.

Analytical Performance and Validation Requirements

During the laboratory validation of EUCAST RAST, core analytical performance indicators, including categorical agreement, very major errors (VMEs; false susceptible results), major errors (false resistant results), and minor errors, must be systematically evaluated. In particular, the evaluation of VMEs is clinically important for sepsis. This is because falsely categorizing a resistant isolate as susceptible may lead to ineffective antimicrobial therapy administration, adversely affecting patient outcomes. Therefore, validation studies should ensure that performance metrics meet the established AST acceptance criteria, typically targeting a categorical agreement of at least 90% and maintaining low VME rates to ensure patient safety and diagnostic reliability.

Recent studies have demonstrated high categorical agreement of EUCAST RAST for Gram-negative pathogens. Shan et al. (2022) reported robust performance of EUCAST RAST for analyzing the susceptibility of Enterobacterales directly from positive blood cultures⁶. A multicenter evaluation by Bianco et al. (2022) confirmed the acceptable agreement of EUCAST RAST across extended reading times⁷. Ekwall-Larson et al. (2023) demonstrated reliable early (4-hour) analytical performance of EUCAST RAST with strong concordance to conventional AST⁸.

Cardot Martin et al. (2022) reported that RAST implementation for analyzing Gram-negative pathogens causing BSIs significantly influenced antimicrobial optimization decisions⁵. Cherkaoui et al. (2022) demonstrated that automated RAST workflows maintained high diagnostic accuracy when integrated with digital systems⁹. Herroelen et al. (2022) validated RAST using automated WASPLab® platforms, confirming reproducibility in high-throughput settings².

Studies during 2024-2025 further support the analytical reliability of RAST. Taysi et al. (2024) used RAST for evaluating the susceptibility of carbapenemase-producing and extended-spectrum beta-lactamase (ESBL)-producing *E. coli* and *K. pneumoniae*, demonstrating strong agreement rates and reporting potential benefits in mitigating mortality with early therapeutic optimization¹⁰. Tian et al. (2025) reported that the categorical agreement rates of RAST were 98%–100% at 6–8 hours for key Gram-negative pathogens¹¹. Aparicio-Calvente et al. (2026) demonstrated that automated digital imaging integration improved the readability and consistency of RAST in early zone interpretation¹². Thus validated EUCAST RAST achieves performance metrics compatible with safe clinical implementation.

Importance of Local Laboratory Validation

Although multicenter evidence supports the analytical reliability of EUCAST RAST, each laboratory must conduct local validation before implementing routine clinical reporting to ensure performance consistency under site-specific conditions. RAST results may be influenced by several pre-analytical variables, including the type of blood culture system, time to positivity, bacterial inoculum density, agar quality and storage conditions, incubation conditions, and overall workflow timing. Yıldız et al. (2025) demonstrated variability in RAST performance across different blood culture platforms, underscoring the need for site-specific validation prior to clinical implementation¹. Additionally, local antimicrobial resistance (AMR) epidemiology significantly affected interpretive reliability. Cerrudo et al. (2023) demonstrated that EUCAST RAST breakpoints can facilitate early inference of β -lactam resistance mechanisms in Enterobacterales. However, the authors emphasized that regional AMR patterns must be considered during implementation¹³. The time constraints associated with the manual implementation of the EUCAST RAST method can be overcome through the use of fully automated systems and digital imaging platforms (e.g., WASPLab®). Herroelen et al. (2022) demonstrated that these automated workflows showed high correlation with the standard disk diffusion method and validated their integration into routine laboratory workflows². For example, in a region with a high prevalence of OXA-48-, NDM-, or KPC-type carbapenemases, a laboratory should include not only standard strains but also local clinical isolates harboring these specific resistance genes when constructing its RAST validation panel. This is critically important for verifying the performance of the method against enzymes that may exhibit low hydrolytic activity and can therefore be more difficult to detect, such as OXA-48¹⁵. To resolve uncertain early readings, Jonasson et al. (2023) proposed extended incubation (16–20 hours) as a complementary strategy¹⁴. Laboratories must establish

clear reporting algorithms to avoid premature therapeutic decisions based solely on results falling within the ATU range. EUCAST indicates that interpretation of results falling within the ATU range is potentially unreliable and recommends that such results should not be reported; instead, incubation should be continued and the test reassessed at later time points. To prevent clinical errors arising from these borderline results, laboratories should establish systematic reporting algorithms aligned with the guideline, which is essential for the safe implementation of the method^{3,14}.

Clinical Impact on Sepsis Management

Rapid availability of phenotypic susceptibility data enables early de-escalation or escalation of antimicrobial therapy. Cardot Martin et al. (2022) revealed that RAST implementation led to therapeutic modifications in patients with several BSIs caused by Gram-negative pathogens⁵. According to Taysi et al. (2024), RAST integration improved the early management of ESBL/carbapenemase-producing pathogen infections¹⁰. Although mortality outcomes depend on various factors, early therapeutic optimization is consistent with the established principles of sepsis management.

Rapid susceptibility reporting strengthens antimicrobial stewardship interventions. Strubbe et al. (2023) reported that the clinical value of RAST increased when integrated into stewardship workflows in a tertiary hospital¹⁶. Rapid susceptibility results enable earlier optimization of empirical therapy, thereby facilitating a timely switch to narrower-spectrum agents. This is consistent with the findings reported by Berinson et al. (2021) regarding earlier transition to optimal therapy, while also reducing unnecessary carbapenem exposure and strategically supporting hospital antimicrobial stewardship (AMS) programs¹⁷.

Recent implementation studies suggest potential reductions in hospital length of stay and antibiotic usage when RAST is incorporated into structured clinical pathways^{5,10}. Automated workflows further streamline laboratory operations and reduce turnaround time^{9,12}.

Automation enhances reproducibility and scalability. Cherkaoui et al. (2022) demonstrated the feasibility of fully automated RAST interpretation⁹. Aparicio-Calvente et al. (2025) reported that digital imaging systems improved the precision of early reading interpretation¹². Automation reduces interobserver variability and supports standardized reporting, which is critical for interpreting the less distinct early readings.

Rapid phenotypic susceptibility testing plays a critical role in mitigating AMR by minimizing unnecessary exposure to broad-spectrum antibiotics, facilitating early de-

escalation to targeted antimicrobial therapy, enabling the timely identification of multidrug-resistant organisms, and improving the overall quality and timeliness of resistance surveillance data. The 2025–2026 EUCAST updates on resistance detection and QC further strengthen the structural framework required for the harmonized global implementation of RAST^{3,4}. A rigorous laboratory-validated RAST is a scalable and adaptable diagnostic strategy that can be effectively implemented across diverse healthcare systems, including resource-limited settings where access to advanced molecular diagnostic technologies may be limited.

Limitations and Implementation Considerations

Although the EUCAST RAST method provides rapid data for sepsis management, it also has several important limitations at the stage of clinical integration. First, its analytical performance is sensitive to pre-analytical variables such as bacterial inoculum density, the brand of the blood culture system, and the incubation atmosphere^{3,5}. From an operational perspective, the requirement that plates be read within predefined time windows (± 5 minutes), which may extend beyond routine working hours, poses a significant challenge for laboratory staffing and workflow management. In addition, borderline results falling within the Area of Technical Uncertainty (ATU) frequently require an additional 16–20 hours of incubation, which may reduce the time advantage of rapid reporting. Finally, because the method has been validated for only a limited number of pathogen–antibiotic combinations, confirmation of results by standard AST methods remains necessary^{1,5,6,16}. Currently, the RAST protocol is primarily validated for specific common pathogens, limiting its application for rare isolates or specific antibiotic combinations³.

EUCAST RAST in the Context of Rapid AST Technologies

EUCAST RAST is positioned as a practical rapid phenotypic alternative among diagnostic technologies used in sepsis management. Molecular panels such as BioFire BCID2 can provide genotypic results from positive blood cultures in about 1 hour, but they target only a predefined set of resistance genes and are associated with higher costs than conventional workflows¹⁸. Similarly, automated phenotypic systems such as the Accelerate Pheno™ system provide rapid results, but they require dedicated instrumentation and workflow investment^{19,20}. In contrast, EUCAST RAST is based on standard disk diffusion methodology and enables phenotypic susceptibility reporting within 4–8 hours directly from positive blood cultures. Although challenges remain, including limited validated organism–antimicrobial combinations and management of results within the area of technical uncertainty (ATU), RAST may represent a scalable and

lower-cost phenotypic option, particularly for laboratories already using conventional disk diffusion methods^{3,4,14}.

Future Perspectives: The Expanding Role of the Clinical Microbiologist in Rapid AST Implementation

The future development of rapid antimicrobial susceptibility testing (AST), including EUCAST RAST, will not be determined solely by technological innovation but by the expanding clinical and interpretive role of the clinical microbiologist. As rapid phenotypic testing shortens the time between blood culture positivity and susceptibility reporting, the microbiologist transitions from a passive diagnostic provider to an active participant in real-time clinical decision-making.

The implementation of EUCAST RAST requires rigorous local validation, continuous quality control (QC) monitoring, and strict adherence to standardized methodological criteria^{3,4}. In this context, the clinical microbiologist assumes primary responsibility for designing validation panels that reflect local antimicrobial resistance epidemiology, including carbapenemase-producing Enterobacterales and other multidrug-resistant organisms^{1,13}. Selection of resistant isolates, evaluation of categorical agreement, and monitoring of VMEs are not purely technical exercises but critical patient safety measures, particularly in septic patients where false susceptible results may have fatal consequences.

As automation and digital imaging platforms become increasingly integrated into laboratory workflows, the microbiologist's role evolves toward oversight of algorithmic interpretation and analytical governance. Studies have demonstrated that automated RAST workflows and digital zone reading systems improve reproducibility and reduce interobserver variability^{2,9,12}. However, early inhibition zones at 4–6 hours may be less sharply defined, requiring expert interpretation in cases falling within the area of technical uncertainty (ATU). The microbiologist must therefore establish clear reporting algorithms, determine when extended incubation (16–20 hours) is required, and prevent premature therapeutic decisions based on borderline results¹⁴.

Beyond analytical oversight, the clinical microbiologist plays a pivotal role in integrating rapid AST results into antimicrobial stewardship frameworks. Evidence suggests that the clinical impact of RAST increases significantly when embedded within structured stewardship pathways¹⁶. Direct communication of early susceptibility data to infectious diseases specialists, participation in multidisciplinary sepsis rounds, and real-time therapeutic recommendations enhance the translation of laboratory data into optimized antimicrobial therapy. In this model, the microbiology laboratory functions not merely as a diagnostic unit but as a strategic component of sepsis management infrastructure.

Current automated RAST systems described in the literature (e.g., WASPLab® and BD Kiestra™) are primarily based on digital image analysis and expert-system algorithms^{9,12}. Although these systems automate image acquisition and preliminary interpretation, as also emphasized by Aparicio-Calvente et al. (2025), fully autonomous AI-based analysis tools are still regarded more as a future perspective than as a component of routine clinical practice¹². Nevertheless, technological scalability does not eliminate the need for expert clinical interpretation. The microbiologist will remain essential for validating new antimicrobial agents, adapting RAST to evolving EUCAST breakpoint updates and ensuring harmonized implementation across diverse healthcare systems^{3,4}.

In resource-limited settings, where access to molecular diagnostics may be constrained, the microbiologist's expertise in standardized phenotypic methods becomes even more critical. Scalable, cost-effective rapid AST models supervised by trained clinical microbiologists may substantially improve early therapeutic optimization while preserving diagnostic reliability.

In summary, the future of rapid AST is inseparable from the expanding clinical leadership of the microbiologist. Through validation oversight, interpretive expertise, stewardship collaboration, and technological governance, the clinical microbiologist remains the central actor in ensuring that rapid susceptibility testing translates into safe, effective, and resistance-conscious patient care.

Conclusions

EUCAST RAST represents a significant advancement in the rapid phenotypic management of BSIs and sepsis. Recent studies have consistently demonstrated high categorical agreement and acceptable error rates when laboratories adhere to standardized protocols and conduct rigorous local validation. The clinical impact of validated RAST includes early therapeutic optimization, strengthened antimicrobial stewardship, and improved healthcare resource utilization. However, speed must not compromise accuracy. Laboratory-specific validation, alignment with EUCAST QC standards, and integration into clinical decision-support frameworks are essential to ensure safe and effective implementation. Validated EUCAST RAST balances both rapid diagnostics and analytical precision, providing clinical benefits.

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