BIOMÉRIEUX

A **DEFINITIVE GUIDE**

FAST ANTIMICROBIAL SUSCEPTIBILITY TESTING

Alternative Methods for Improving Antimicrobial Therapy

Contents

Fast antimicrobial susceptibility testing drives faster results

Bloodstream infections (BSIs), commonly caused by bacterial pathogens, are one of the leading causes of mortality from infections, leading to approximately 250,000 deaths in North America and Europe each year.[1] Antimicrobial susceptibility testing (AST) is critical to the effective treatment of BSIs. AST determines the level at which a particular antimicrobial inhibits the growth of the bacteria or fungi that is causing an infection.[2] However, AST often takes time, forcing clinicians to prescribe empiric, broad-spectrum antimicrobials before they have definitive test results. This leads to overuse and misuse of antimicrobials, one of the leading drivers of antimicrobial resistance (AMR).

Over the past decade, novel fast AST methods have been developed that significantly reduce the time to pathogen identification and susceptibility profile determination. Many of these technologies allow for AST directly from positive blood cultures, eliminating the hours- or days-long wait time. This guide explains why AST is important, provides an overview of the sepsis crisis, delineates the advantages of fast AST compared to conventional methods, describes phenotypic and genomic methods, and reviews new methods.

Why is *in vitro* susceptibility testing so important?

In vitro susceptibility testing acts as a vital aid in clinical settings, helping clinicians select the most appropriate antimicrobial therapy for individual patients, monitor the evolution of microbial resistance, and update empiric therapeutic strategies.

Providing clinical guidance[3]

The primary purpose of routine *in vitro* AST in the clinical microbiology lab is to guide clinicians in selecting antimicrobial therapy for the treatment of individual patients. Susceptibility testing is performed on bacterial and fungal strains isolated from an individual patient and presumed to be the etiology of their infection. Clinicians use this data along with other available clinical information (e.g., site of infection, severity of infection, immune status of patient, co-morbidities, organ function) to select the optimal therapeutic agent to treat the patient's infection.[4] Because of time limitations associated with AST, antimicrobial therapy is often initiated prior to obtaining susceptibility testing results. In these instances, the susceptibility testing outcomes confirm the appropriateness of the empiric therapy and/or indicate appropriate alternative antimicrobials.

Tracking antimicrobial resistance[4]

Conducting periodic statistical analysis of the accumulated resistance levels per species, type of specimen, and patient establishes evidence-based guidelines for the initial empiric choice of antimicrobial therapy. The antimicrobial resistance pattern—by unit, healthcare setting, locality, region, and/or country—also guides antimicrobial formulary decisions. Detailed statistical analysis enables the detection of new resistance patterns or possible outbreaks caused by multi-drug resistant organisms (MDROs), especially in hospital or long-term care settings. This may indicate the need for implementation or change of infection control practices.

WHY IS *IN VITRO* SUSCEPTIBILITY TESTING SO IMPORTANT?

Reducing delays to prevent life-threatening patient outcomes

For patients with serious infections, shortening time to effective antimicrobial therapy (TTET) reduces significant negative outcomes.[5,6] This is most often observed in gram-negative resistant pathogens.[7]

Research shows that patients with delayed TTET have:[8]

- Longer durations of antimicrobial therapy
- Increased lengths of stay
- Higher costs
- Decreased likelihood of discharge to home (versus another healthcare facility)
- Increased mortality

Research also shows that one of the best ways to improve TTET is to involve microbiology lab professionals in antimicrobial stewardship interventions.[9]

Measures of antimicrobial susceptibility

The basic measurement of susceptibility testing is the minimum inhibitory concentration (MIC), which is defined as the lowest concentration of a range of antimicrobial dilutions that inhibits the visible growth of bacteria or fungi within a defined period.[10] The MIC is determined under standardized conditions (e.g., incubation temperature, duration, and inoculum size), which are defined by the Clinical and Laboratory Standards Institute (CLSI), Food and Drug Administration (FDA), International Organization for Standardization (ISO), or European Committee on Antimicrobial Susceptibility Testing (EUCAST).[11-14]

The MIC values of different antimicrobials tested in a patient sample are then compared against clinical breakpoints, which are used to determine whether a species of bacteria is susceptible or resistant to an antimicrobial. Clinical breakpoints are developed through a detailed examination of MIC data and distributions, resistance data and mechanisms, and analysis of pharmacokinetic and pharmacodynamic properties of the antimicrobials.[15]

Based on the clinical breakpoints, a patient-specific pathogen will be categorized as being:[16]

S—Susceptible, meaning the antimicrobial is likely to be therapeutically successful at a standard dosing regimen.

SDD—Susceptible dose-dependent, refers to susceptibility that depends on the dosing regimen used by the patient.

I—Susceptible with increased exposure, meaning the antimicrobial is likely to be therapeutically successful with an adjusted dosing regimen.

R—Resistant, meaning the antimicrobial is likely to be therapeutically ineffective, even at high doses.

Sepsis: a danger to patients and antimicrobial resistance

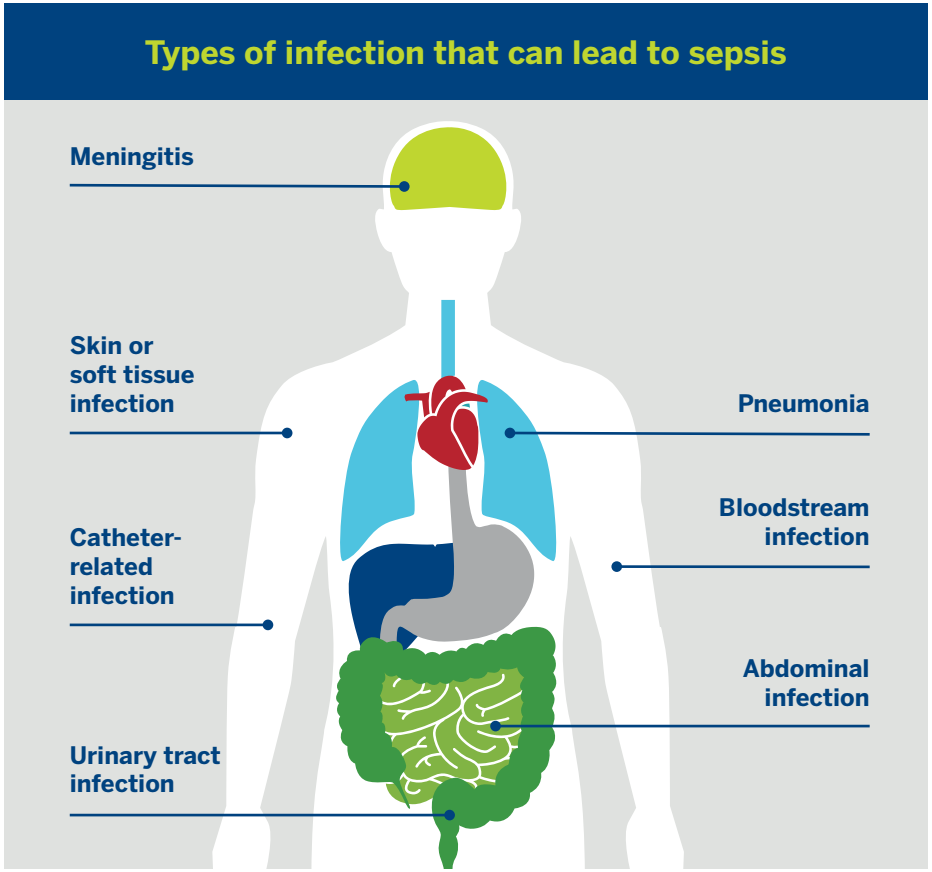
Sepsis is one of the most frequently occurring and challenging infectious diseases that demand effective AST. Worldwide there were 48.9 million cases of sepsis in 2017 leading to 11 million deaths, which represented 20% of all global deaths that year.[17] Roughly 85% of sepsis-related deaths occurred in low- and middle-income countries (LMICs).[17] In US hospitals, sepsis is the third most common cause of death.[18] Each year sepsis affects 1.7 million people nationwide, leads to nearly 270,000 deaths, and costs an estimated $62 billion in hospitalizations and skilled nursing care.[18,19]

Sepsis is alarmingly frequent: one in every three patients who die in a hospital has sepsis.[20] More than 87% of sepsis cases originate in the community versus in a hospital setting.[21] Additionally, delays in time to antimicrobial treatment increase mortality.[18]

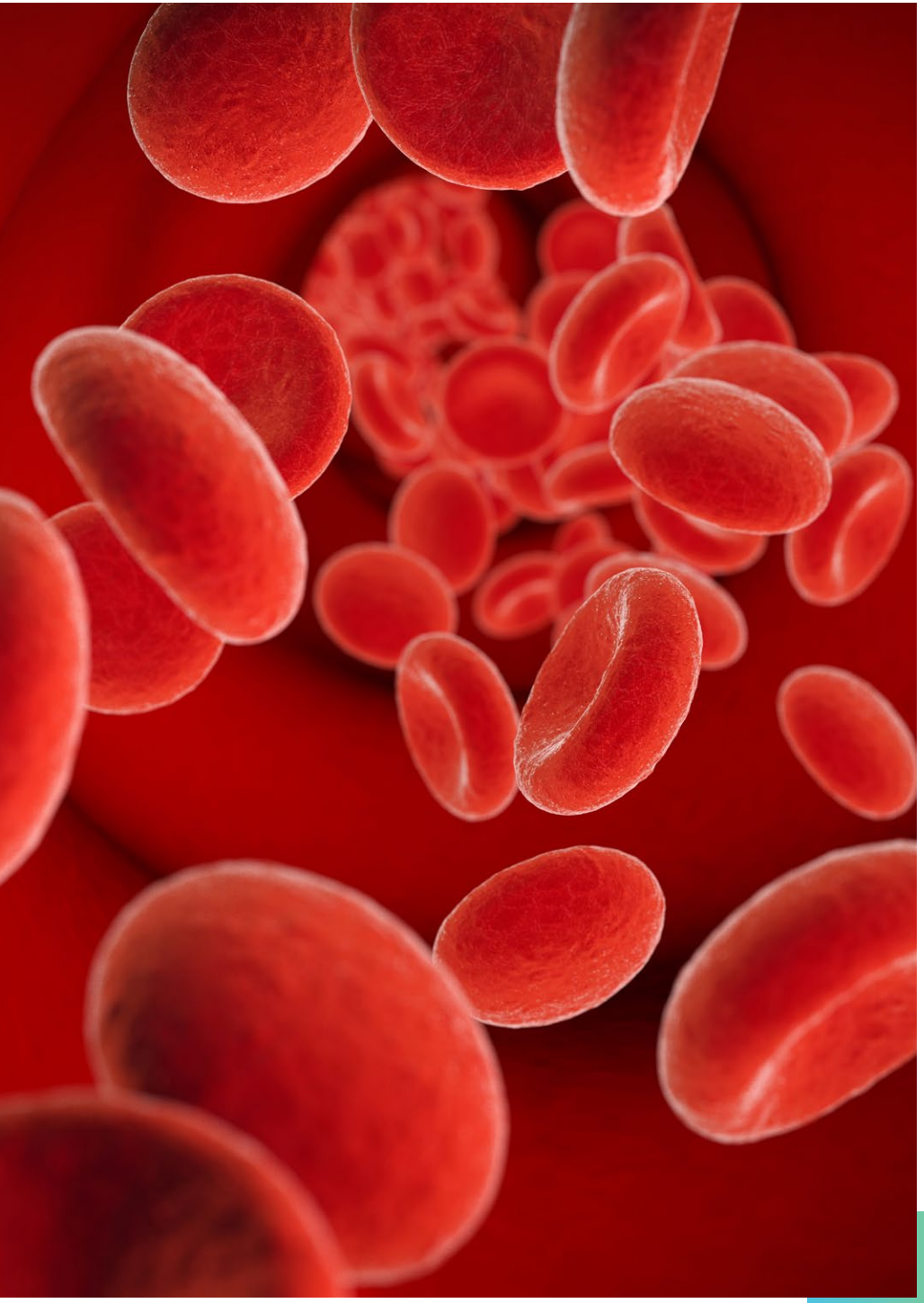
Unfortunately, sepsis can be difficult to diagnose because its symptoms mirror those of other conditions. Common signs and symptoms include:[20]

- High heart rate or low blood pressure
- Extreme pain or discomfort
- Fever, shivering, or feeling very cold
- Clammy or sweaty skin
- Shortness of breath
- Confusion or disorientation

Bacteria cause most cases of sepsis, although some viral, fungal, or parasitic infections can also lead to sepsis.[22] Treatment relies on early use of antimicrobials to address the causative pathogen, but it may also include maintaining blood flow to organs via intravenous fluid, or, for low blood pressure, the application of vasopressors.[23] Unfortunately, there is no single laboratory test that can diagnose sepsis.



While sepsis patients must receive antimicrobial treatment as quickly as possible, there is an inherent dilemma—short-term broad-spectrum antimicrobials used empirically before susceptibility testing is completed may lead to greater antimicrobial resistance.[24] That means clinicians must strike the right balance in treatment between short-term, patient-focused efforts to improve outcomes with longer-term, community-wide efforts to prevent AMR. This frequently translates into using broad-spectrum empiric antimicrobial treatment and narrow-spectrum antimicrobials once susceptibility testing is available. Given the scope and complexity of sepsis, fast AST has become a diagnostic imperative.



Advantages of fast AST for bloodstream infections

New AST technologies deliver susceptibility results more quickly than conventional options like automated/manual broth microdilution, disk diffusion, and antimicrobial gradient methods. With fast AST technologies, the time between the inoculation of a bacterial strain with different antimicrobial concentrations and results can be significantly shorter.[25] Additionally, some fast AST technologies use sensitive (colorimetric or fluorimetric) optical systems rather than conventional visual inspection to determine bacterial growth inhibition, which improves test accuracy.

Most fast AST systems rely on expert software to carry out results interpretation. Automated systems express test results as quantitative MICs, qualitative breakpoints, or both. This minimizes the uncertainty in results interpretation characterized by manual methods. Test reports include both individual patient outcomes, which are designed to communicate AST results to treating clinicians, and historical data sets, which are used for epidemiology and public health management.

Ultimately, automated and fast AST methods provide reliable, quantitative and qualitative AST results. These instruments often require less hands-on time and provide results for many pathogen-antimicrobial compound combinations more quickly than conventional methods. In addition, the integration of software modules supports the interpretation and sharing of treatment-relevant data.

Proven results

Some studies highlight evidence that fast AST reduces the time to susceptibility results and enables clinicians to optimize antimicrobial therapy faster.

Fast AST for gram-negative bloodstream infections[26]

This study compared fast AST technologies (in this case the Alfred 60AST System) to conventional testing for gram-negative bloodstream infections. The mean total time from positive blood culture to identification and fast susceptibility results was approximately six hours for the fast testing and 20 hours for the conventional testing. Results for fast testing were communicated to clinicians on the same working day. The study showed that faster testing led to the earlier use of effective antimicrobials and earlier discontinuation of aminoglycosides used in empiric therapy. It also resulted in significant reductions in the median time from blood culture collection to communication of AST results—from 55.2 hours in the conventional group to 33.1 hours in the fast group. In patients on ineffective empiric antimicrobials, effective treatment based on AST results started a median of 17.5 hours earlier in the fast group compared to the conventional group (P=0.036). Combination therapy with aminoglycosides was stopped earlier for patients in the fast AST group versus the conventional group (31.5 versus 53.7 hours respectively, P=0.005).

Fast AST for gram-negative bacilli (GNB) bloodstream infections[27,28]

This randomized study compared standard-of-care (SOC) to fast AST using the Accelerate PhenoTest® BC kit. The study concluded that fast organism identification and phenotypic AST led to faster changes in antimicrobial therapy for gram-negative BSIs. All patients in both arms underwent prospective audits and feedback by institutional antimicrobial stewardship programs (ASPs). The time to first antimicrobial modification was faster with the PhenoTest BC method (8.6 hours) compared to SOC (14.9 hours, P=0.02). Additionally, gram-negative antimicrobial changes were faster with PhenoTest BC (17.3 hours) compared to SOC (42.1 hours). Time to first antimicrobial escalation was significantly faster with PhenoTest BC (18.4 hours) compared to SOC (61.7 hours) for antimicrobial-resistant BSIs, but time to de-escalation did not differ statistically.

New advances in phenotypic and genotypic AST methods

There are two primary types of fast AST: phenotypic and genotypic. Traditional AST is phenotypic, which delivers comprehensive, reliable results that often require significant time to obtain. That is because before starting the test, isolated colonies of the organism must be isolated from agar plates post-incubation, which can take 8–24 hours. Disk diffusion, agar dilution, and broth microdilution are the most common phenotypic methods. Fast phenotypic methods can reduce the time to initial result but do have some limitations. Most notably, they require monomicrobial cultures and may lead to inaccurate or delayed reporting for polymicrobial cultures.[29]

Genotypic methods refer to fast molecular tools for quantifying and profiling bacterial and fungal pathogen genes that use DNA-based, amplification-based, or sequencing-based molecular approaches.[30] The most recognized genotypic amplification techniques for detecting antimicrobial resistance include polymerase chain reaction (PCR), DNA microarray, DNA chips, and loop-mediated isothermal amplification (LAMP).[31] Genotypic methods test directly from positive blood cultures without the need for subculturing isolated bacterial colonies, which saves significant time. In addition to detecting resistance genes, they also provide organism identification and tend to be both sensitive and specific.[31]

However, while genotypic testing enables more accurate results and faster time to alter antimicrobial therapy, it may not accurately predict a full susceptibility profile.[32] For example, positive or negative PCR results do not always translate into phenotypic antimicrobial resistance or susceptibility (e.g., non-carbapenemase producing, carbapenem-resistant Enterobacterales would not be identified by available genotypic methods).

Fast AST technologies: a review of the current landscape

Phenotypic and genotypic fast AST technologies are continuously emerging and evolving. Each provides unique improvements in pathogen identification and AST and, most importantly, potential improvements in TTET. But they also come with limitations: some still require incubation of blood cultures, some reduce the speed of throughput, many impose space constraints, and most currently involve increased instrument and reagent costs. It is important to recognize that no single test replaces others—each must be evaluated for how well it fits with existing lab standards, protocols, staffing, and budgets.

Direct disk diffusion

Direct disk diffusion uses blood directly from a positive blood culture bottle, which enables the testing to be completed in as few as four hours following the EUCAST standard and eight hours following the CLSI standard.[33-36]

Microfluidic systems

A number of new phenotypic technologies use microfluidics and microscopy to detect bacteria in blood cultures at a single-cell level. Key advantages of these systems are they use small sample quantities, have high detection sensitivity, and significantly reduce analysis time.

Flow cytometry

AST via flow cytometry is performed directly from positive blood culture broth, making it significantly faster to early results. Essentially, each antimicrobial agent is prepared the same way as a flow cytometry panel but with the concentrations of a microdilution test. The software then interprets the data immediately and reports MICs that meet EUCAST and CLSI standards.

Volatile organic compounds

Colorimetric sensor arrays, another new fast AST technology, use sensory technology to detect the release of volatile organic compounds (VOCs), which are emitted by bacteria during growth. The sample preparation from positive blood culture broth can be completed in minutes and AST results can be delivered in hours, with the average time to result being 3–7.5 hours, depending on the specific organism/antimicrobial combination.[37]

Fast AST: driving measurable improvements in antimicrobial therapy

Patients with BSIs, particularly sepsis, need accurate insight into pathogen identification and resistance patterns more quickly so empiric antimicrobial therapy can be replaced with optimized, definitive antimicrobials. Fast AST methods have expanded the arsenal of susceptibility testing options and generally achieve faster AST results than conventional AST methods, enabling clinicians to make informed decisions more quickly. Some fast ASTs save time by imaging the growth of a single bacteria or small groups of bacteria; others use novel technologies to detect specific signals of bacterial growth, such as VOCs. In this guide, we have provided lab professionals and clinicians with an overview of current fast AST technologies. As additional novel fast technologies emerge, the real goal of obtaining a pathogen's susceptibility profile prior to the initiation of antimicrobial therapy will come closer to fruition.

Resources

bioMérieux is committed to helping lab professionals and clinicians better understand AST standards, best practices, and technology options. We offer several educational resources related to AST and fast AST.

VITEK® REVEAL™ offers fast AST results to effectively and efficiently manage bloodstream infections.

Visit Page

go.biomerieux.com/vitek-reveal/system

Antimicrobial susceptibility testing of bacteria and fungi brochure

View Brochure

www.biomerieux.com/content/dam/biomerieux-com/medical-affairs/educational-booklets/booklet_antimicrobial-susceptibility-testing-educational-booklet-offered-by-biomerieux.pdf

References

1. Goto, M., et. al. Overall burden of bloodstream infection and nosocomial bloodstream infection in North America and Europe. *Clin Microbiol & Infect*. 2013;19(6):501-509. doi: 10.1111/1469.0691.12195
2. Testing.com. Antimicrobial susceptibility testing. 2020.
3. Smith, K. et. al. Rapid susceptibility testing methods. *Clin Lab Med*. 2019;39(3):333-344. doi: 10.1016/j.cll.2019.04.001
4. Reller, L., et. al. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. *Clin Infect Dis.* 2009;49(11):1749-1755. doi: 10.1086/647592
5. Evans, R., et. al. Modifiable healthcare factors affecting 28-day survival in bloodstream infection: a prospective cohort study. B*MC Infect Dis.* 2020;20:545. doi: 10.1186/s12879-020-05262-6
6. Bonine, N., et. al. Impact of delayed appropriate antibiotic therapy on patient outcomes by antibiotic resistance status from serious gram-negative bacterial infections. *Am J of Clin Sci.* 2018;357(2): 103-110. doi: 10.1016/j.amjms.2018.11.009
7. Perez, K., et. al. Integrating rapid diagnostics and antimicrobial stewardship improves outcomes in patients with antibiotic resistant, gram-negative bacteremia. *J Infect.* 2014;69(3):216-225. doi: 10.1016/j.jinf.2014.05.005
8. Lodise, T., et. al. Antimicrobial resistance or delayed appropriate therapy—does one influence outcomes more than the other among patients with serious infections due to Carbapenem-resistant versus Carbapenem-susceptible enterobacteriaceae? *Open Forum Infect Dis.* 2019;6(6):ofz194. doi: 10.1093/ofid/ofz194
9. Beall, A. How labs can bolster antimicrobial stewardship. T*oday's Clinical Lab*. 2020. Online article.
10. Andrews, J. Determination of minimum inhibitory concentrations. *J Antimicrob Chemoth*. 2001;48(Suppl 1):5-16. doi: 10.1093/jac/48suppl_1.
11. Clinical Laboratory Standards Institute. CLSI-ISO 20776-2:2021 Clinical laboratory testing and in vitro diagnostic test systems—susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices—part 2. https://clsi.org/standards/products/iso-documents/documents/iso-20776-2-2021/
12. US Food and Drug Adminstration. Antimicrobial resistance and medical devices. 2023. https://www.fda.gov/medical-devices/products-and-medical-procedures/antimicrobial-resistance-and-medical-devices#FDAInvolve
13. International Standardization Organization. ISO20776-2:2021: Clinical laboratory testing and in vitro diagnostic test systems. 2021. https://www.iso.org/standard/79377.html
14. ESCMID—European Committee on Antimicrobial Susceptibility Testing. Antimicrobial susceptibility testing. Website. https://www.eucast.org/ast_of_bacteria#:~:text=EUCAST%20antimicrobial%20susceptibility%20testing%20is%20performed%20with,whether%20the%20organism%20is%20categorised%20as%20susceptible
15. Patel, J., et. al. Updating breakpoints in the United States: a summary from the ASM Clinical Microbiology Open 2022. *Clin Microbiol*. 2023. doi: 10.1128/jcm.01154-22
16. US Food and Drug Administration. FDA-recognized antimicrobial susceptibility test interpretive criteria. 2023. https://www.fda.gov/drugs/development-resources/fda-recognized-antimicrobial-susceptibility-test-interpretive-criteria#6633ff5d5fb5f
17. World Health Organization. Sepsis fact sheet. July 19, 2023. https://www.who.int/news-room/fact-sheets/detail/sepsis
18. Association of American Medical Colleges. Sepsis is the third leading cause of death in US hospitals. But quick action can save lives. AAMC News. 2023.
19. National Institute of General Medical Sciences. Sepsis fact sheet. https://www.nigms.nih.gov/education/fact-sheets/Pages/sepsis.aspx#:~:text=Sepsis%20is%20a%20serious%20condition,270%2C000%20die%20as%20a%20result.
20. Centers for Disease Control and Prevention. What is sepsis? Website. https://www.cdc.gov/sepsis/what-is-sepsis.html
21. Sepsis Alliance. Septic shock. Website. https://www.sepsis.org/sepsisand/septic-shock/#:~:text=People%20are%20more%20likely%20to,can%20come%20on%20very%20suddenly
22. Cleveland Clinic. Sepsis. Health Library, Diseases and Conditions. 2023. https://my.clevelandclinic.org/health/diseases/12361-sepsis
23. Evans, L., et. al. Surviving Sepsis Campaign: International guidelines for management of sepsis and septic shock 2021. *Crit Care Med.* 2021;49(11):e1063-e1143. doi: 10.1097/CCM.0000000000005337
24. Strich, J., et. al. Considerations for empirical antimicrobial therapy in sepsis and sepsis shock in an era of antimicrobial resistance. *J Infect Dis.* 2020;222(Suppl 2):S119-S131. doi: 10.1093/infdis/jiaa.221
25. Banerjee, R., et. al. Rapid antimicrobial susceptibility testing methods for blood cultures and their clinical impact. *Front Med*. 2021;8: 635831. doi: 10.3389/fmed.2021.635831
26. Anton-Vazquez, V., et. al. Impact of rapid susceptibility testing on antimicrobial therapy and clinical outcomes in gram-negative bloodstream infections. *J Antimicrob Chemother*. 2022;77:771-781. doi: 10.1093/jac/dkab449
27. Banerjee, R., et. al. Randomized trial evaluating clinical impact of rapid identification and susceptibility testing for gram-negative bacteremia: RAPIDS-GN. *Clin Infect Dis.* 2021;73(!);e39-46. doi:10.1093/cid/ciaa528
28. Christiansen, A., et. al. Impact of a laboratory-developed phenotypic rapid susceptibility test directly from positive blood cultures on time to narrowest effective therapy to patients with gram-negative bacteremia: a prospective randomized trial. *OFID*. 2022;1-8. doi: 10.1093/ofid/ofac.347
29. Garrett, E., et. al. Recent advances in direct blood culture phenotypic antimicrobial susceptibility testing. *Clinical Microbiology Newsletter*. 2022;44(23):209-216. doi: 10.1016/j.clinmicnews.2022.11.005
30. Salam, A., et. al. Conventional methods and future trends in antimicrobial susceptibility testing. *Saudi J Biol Sci*. 2023;30(3):103582. doi: 10.1016/j.sjbs.2023.103582
31. Khan, Z. et. al. Current and emerging methods of antibiotic susceptibility testing. *Diagnostics*. 2019;9(2):49. doi: 10.3390/diagnostics9020049
32. Bard, J., et. al. Why can't we just use PCR? The role of genotypic versus phenotypic testing for antimicrobial resistance testing. *Clin Microbiol NewsI*. 2018;40(11):87-95. doi: 10.1016/j.clinmicnews.2018.05.003
33. European Committee on Antimicrobial Susceptibility Testing. Rapid AST directly from blood culture bottles. EUCAST Website. 2020.
34. Webber, D., et. al., Stop waiting for tomorrow: disk diffusion performed on early growth is an accurate method for antimicrobial susceptibility testing with reduced turnaround time. *J Clin Microbiol*. 2022;60(5):1-10. doi: 10.1128/jcm.03007-20
35. Clinical and Laboratory Standards Institute. CLSI M100: Performance standards for antimicrobial susceptibility testing. 34th edition. 2024.
36. Schuetz, A., et. al. Implementation of the CLSI method for disk diffusion testing from positive blood cultures. Clinical and Laboratory Standards Institute Website. 2022.
37. Couchot, J., et. al. Evaluation of the REVEAL® rapid AST system to assess the susceptibility of Pseudomonas aeruginosa from blood cultures. *Eur J Clin Microbiol Infect Dis.* 2023;42:359-363. doi: 10.1007/s10096-023-04556-2

A major player in *in vitro* diagnostics for more than 60 years, bioMérieux has always been driven by a pioneering spirit and unrelenting commitment to improve public health worldwide.

Our diagnostic solutions bring high medical value testing to healthcare professionals, providing them with the most relevant and reliable information, as quickly as possible, to support treatment decisions and better patient care.

bioMérieux's mission entails a commitment to support medical education, by promoting access to diagnostic knowledge for as many people as possible. Focusing on the medical value of diagnostics, our collection of educational booklets aims to raise awareness of the essential role that diagnostics test results play in healthcare decisions.

Other educational booklets are available.
Consult your local bioMérieux representative or visit
biomerieux.com/en/education/educational-booklets

BIOMÉRIEUX

Contact Us

bioMérieux, Inc.
515 Colorow Drive
Salt Lake City, UT 84108
Tel: (801) 736 6354
Fax: (800) 968 9494
biomerieux-usa.com
biofiredx.com