Volume 11, November 2025

CLSI Subcommittee on Antimicrobial Susceptibility Testing

CLSI AST News Update

Janet A. Hindler, MCLS, MT(ASCP), F(AAM), Editor
Audrey N. Schuetz, MD, MPH, D(ABMM), Editor

This biannual CLSI AST News Update highlights current issues related to antimicrobial susceptibility testing (AST) and reporting.

Inside This Issue:

CLSI and the AST Subcommittee Meetings

1. Content from past meetings can be found **here.**
2. Save the date for the next meetings:
   - January 22–27, 2026 | Tempe, AZ
   - May 30–June 2, 2026 | Chicago, IL

What does the CLSI AST Subcommittee do?

The first edition of the CLSI AST News Update (Vol 1, Issue 1, Spring 2016) described details about the organization and operation of the CLSI AST Subcommittee.

- You can access that Newsletter **here.**
- To learn more about upcoming or past meetings, click **here.**
- CLSI posts meeting minutes and summaries for public access **here.**
- For a quick overview, you can check out a "New Attendee Orientation" video presentation **here.**

Interested in becoming a CLSI volunteer? Learn more here.

Please remember that CLSI AST Subcommittee welcomes suggestions from you about any aspect of CLSI documents, educational materials, or this News Update.

Featured Article

A Clinical Case of *Burkholderia cepacia* Complex and Changes to CLSI Antimicrobial Susceptibility Testing Recommendations

Holly Huse, Harbor-UCLA Medical Center, Torrance, CA
Robert Bowden, Beth Israel Deaconess Medical Center, Boston, MA
Priyanka Uprety, Quest Diagnostics, Clifton, NJ

A 52-year-old patient with a recent diagnosis of acute lymphoblastic leukemia was hospitalized for induction chemotherapy at a tertiary care hospital in the United States. On day 1 of his hospital stay, the patient developed a fever. The patient had recently received medical care in Mexico, including placement of a peripherally inserted central catheter line.

Multiple sets of blood cultures were obtained, and they were all positive for gram-negative rods. A rapid molecular panel was performed, and it was negative for organism identification and resistance gene targets. Upon culture growth, an identification of *Burkholderia multivorans* was made using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). *Burkholderia multivorans* is a member of the *Burkholderia cepacia* complex (BCC). Susceptibility testing was not performed by the laboratory.

Q1. What are the recent changes to CLSI recommendations for BCC organisms?

A1. In 2024, CLSI removed disk diffusion (DD) breakpoints from CLSI M100 for BCC organisms due to poor reproducibility and poor correlation with CLSI reference broth microdilution (BMD).[1] In 2025, CLSI removed minimal inhibitory concentration (MIC) breakpoints from CLSI M100 based on data showing that 2 CLSI reference antimicrobial susceptibility testing (AST) methods, BMD and agar dilution, showed poor correlation.[2] These changes are in alignment with the European Committee on Antimicrobial Susceptibility Testing (EUCAST), which has refrained from publishing DD or MIC breakpoints for BCC because of the poor correlation between AST methods and lack of correlation between AST results and clinical outcomes in patients with cystic fibrosis.[3,4]

Q2. What species are included in the *Burkholderia cepacia* complex?

A2. The *Burkholderia cepacia* complex (BCC) continues to undergo taxonomic review. The 3 most commonly isolated species from human infections are *B. cenocepacia, B. cepacia,* and *B. multivorans.* Other members include *Pararobbsia alpina* (also known as *Burkholderia alpina*), *B. ambifaria, B. anthina, B. arboris, B. catarinensis, B. contaminans, B. diffusa, B. dolosa, B. lata, B. latens, B. metallica, B. pseudomultivorans, B. puraquae, B. pyrrocinia, B. seminalis, B. stabilis, B. stagnalis, B. territorii, B. ubonensis,* and *B. vietnamiensis.*[5-7] *B. paludis* has been included in BCC but is not a validly published name. Of note, *B. gladioli* is also isolated from human infections but is not part of the BCC.

Q3. Should the laboratory routinely provide AST results for BCC organisms?

A3. No, and the laboratory may consider adding the following comment:

"Antimicrobial susceptibility testing is not performed for organisms of the *Burkholderia cepacia* complex due to issues with method accuracy and limited clinical outcome data. Consultation with an Infectious Diseases specialist is highly recommended."

Q4. How should I communicate this change to my clinicians?

A4. As with all decisions regarding AST and reporting, the laboratory should discuss these changes with their antimicrobial stewardship team and other relevant stakeholders in their institution.

A Clinical Case of *Burkholderia cepacia* Complex and Changes to CLSI Antimicrobial Susceptibility Testing Recommendations (*Continued*)

Q5. What is the risk of using the "old" breakpoints and "old" methods for AST of BCC in my laboratory? What if breakpoints for "Other Non-Enterobacterales" or epidemiological cutoff values (ECVs) are used instead?

A5. Disk diffusion and commercial MIC methods, including agar gradient diffusion, demonstrate high rates of errors, both false-susceptible and false-resistant results, when testing BCC and using the previously published BCC breakpoints or if trying to apply alternative breakpoints.[8-11] ECVs are intended only for epidemiological purposes and not to guide therapy, with CLSI M100 noting that wild-type isolates of BCC often exhibit MICs that may exceed what is achievable with routine dosage regimens.[2] Antimicrobial susceptibility test results have on occasion been used in the past to guide lung transplantation in patients with cystic fibrosis. In the most extreme cases, patients might have been denied lung transplant based on reporting of an isolate as highly resistant, with those AST results having been inaccurate due to the issues described here.[12]

Q6. What can be done if a provider insists on obtaining an AST result for a BCC organism?

A6. In the 35th edition of CLSI M100, Table 2B-3 states: "If testing is performed, reference BMD (frozen) is the only reproducible method and laboratories might consider including the comment, "correlation of MIC values with clinical outcome is not known."[2] Testing should only be performed upon request from the antimicrobial stewardship team following their consultation with the provider involved with the patient.

Q7. Is there any guidance for performance of AST for *Burkholderia* species other than BCC?

A7. Testing recommendations for *B. mallei* and *B. pseudomallei* can be found in CLSI M45,[13] as they fall under potential bacterial agents of bioterrorism. Other *Burkholderia* species that are not within the BCC and are not *B. mallei* or *B. pseudomallei* (ie, *B. gladioli*) fall into the category "Other Non-Enterobacterales" for which there are testing recommendations and MIC breakpoints listed in Table 2B-5 in the 35th edition of CLSI M100.[2] However, some caution may be warranted when using this table as the recommendations herein are being reassessed and their applicability to *Burkholderia* species other than BCC has not been systematically studied. If using a commercial AST device, it is important to know if the species in question is included in the manufacturer's instructions for use.

References

1. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 34th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2024.
2. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 35th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2025.
3. European Committee on Antimicrobial Susceptibility Testing. 2013. Antimicrobial susceptibility testing of *Burkholderia cepacia* complex (BCC). EUCAST. Accessed 23 October 2025. https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/General_documents/BCC_susceptibility_testing_130719.pdf
4. Somayaji R, Parkins MD, Shah A, et al. Antimicrobial Resistance in Cystic Fibrosis International Working Group. Antimicrobial Susceptibility testing (AST) and associated clinical outcomes in individuals with cystic fibrosis: A systematic review. *J Cyst Fibros.* 2019;18(2):236-243.
5. Vanlaere E, LiPuma JJ, Baldwin A, et al. *Burkholderia latens* sp. nov., *Burkholderia diffusa* sp. nov., *Burkholderia arboris* sp. nov., *Burkholderia seminalis* sp. nov. and *Burkholderia metallica* sp. nov., novel species within the *Burkholderia cepacia* complex. *Int J Syst Evol Microbiol.* 2008;58(Pt 7):1580-1590.
6. Vanlaere E, Baldwin A, Gevers D, et al. Taxon K, a complex within the *Burkholderia cepacia* complex, comprises at least two novel species, *Burkholderia contaminans* sp. nov. and *Burkholderia lata* sp. nov. *Int J Syst Evol Microbiol.* 2009;59(Pt 1):102-111.
7. Parte AC, Sardà Carbasse J, Meier-Kolthoff JP, Reimer LC, Göker M. List of prokaryotic names with standing in nomenclature (LPSN) moves to the DSMZ. *Int J Syst Evol Microbiol.* 2020;70(11):5607-5612.

A Clinical Case of *Burkholderia cepacia* Complex and Changes to CLSI Antimicrobial Susceptibility Testing Recommendations (*Continued*)

8 Fehlberg LCC, Nicoletti AG, Ramos AC, et al. *In vitro* susceptibility of *Burkholderia cepacia* complex isolates: Comparison of disk diffusion, Etest®, agar dilution, and broth microdilution methods. *Diagn Microbiol Infect Dis.* 2016;86(4):422–427.

9 Wootton M, Davies L, Pitman K, Howe RA. Evaluation of susceptibility testing methods for *Burkholderia cepacia* complex: A comparison of broth microdilution, agar dilution, gradient strip and EUCAST disc diffusion. *Clin Microbiol Infect*. 2020;S1198-743X(20)30708-4.

10 Huse HK, Lee MJ, Wootton M, Sharp SE, LiPuma JJ, Jorth P. Evaluation of antimicrobial susceptibility testing methods for *Burkholderia cenocepacia* and *Burkholderia multivorans* isolates from cystic fibrosis patients. *J Clin Microbiol*. 2021;59(12):e01447-21.

11 Jorth P, Manuel C, McLemore T, et al. Evaluation of antimicrobial susceptibility testing methods for *Burkholderia cepacia* complex isolates from people with and without cystic fibrosis. *J Clin Microbiol.* 2025;63(2):e0148024.

12 Leard LE, Holm AM, Valapour M, et al. Consensus document for the selection of lung transplant candidates: An update from the International Society for Heart and Lung Transplantation. *J Heart Lung Transplant*. 2021;40(11):1349-1379.

13 CLSI. *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria.* 3rd ed. CLSI document M45. Clinical and Laboratory Standards Institute; 2016.

Case Study

Key Insights into *Candida auris* from a Clinical Laboratory Perspective

Amir Seyedmousavi, National Institutes of Health, Bethesda, MD
Romney M. Humphries, Vanderbilt University Medical Center, Nashville, TN
Audrey N. Schuetz, Mayo Clinic, Rochester, MN

Clinical Case

A 69-year-old man from a long-term care facility presented to the emergency department with acute respiratory distress. His history included abdominal aneurysm, bowel resection, chronic respiratory failure requiring ventilatory support, and colonization with carbapenem-resistant *Klebsiella pneumoniae*. On arrival, he was in septic shock and admitted to the ICU. Empiric therapy with ceftazidime-avibactam, vancomycin, and micafungin was started for presumed bacteremia. Possible sources included a sacral ulcer, urinary tract, and lungs. Respiratory, urine, and routine blood cultures were obtained. Initial blood cultures were negative, but sputum grew *K. pneumoniae* and moderate yeast, which was not further identified per laboratory policy. By day 2, the urine culture yielded *Candida (Candidozyma) auris* (50 000 colony-forming units [CFU]/mL). On day 4 of the hospital stay, another set of blood cultures was drawn. Four days later (day 8 of the hospital stay), the blood cultures flagged positive for yeast, which was identified directly from the positive blood culture broth as *C. auris* using the cobas® eplex blood culture identification fungal pathogen (BCID-FP) panel. Species identification was confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS, Bruker Daltronics). Antifungal susceptibility testing (AST) of the blood *C. auris* isolate showed the following minimal inhibitory concentrations (MICs): amphotericin B 0.25 µg/mL, fluconazole 64 µg/mL, and micafungin 0.06 µg/mL. Micafungin therapy escalated from 100 to 150 mg daily, and central catheters were removed. Despite this, the patient's condition deteriorated, resulting in multi-organ failure and death.

Background

C. auris has become a global health threat due to rapid spread and difficult clinical management.[1] First identified in 2009, it is now reported in over 50 countries and often causes healthcare outbreaks.[2,3] High mortality, persistence on surfaces, and resistance to standard disinfectants contribute to nosocomial transmission, particularly among immunocompromised and critically ill patients.[4,5] In the United States, the Centers for Disease Control and Prevention (CDC) classifies *C. auris* as an urgent threat, with 4,514 new cases reported in 2023.[2] Although routine colonization screening is not consistently applied in all settings, the rising incidence of *C. auris* underscores the importance of strengthened surveillance, rapid diagnostic tools, and rigorous infection control measures, including hand hygiene, appropriate personal protective equipment (PPE), patient isolation, and use of US Environmental Protection Agency (EPA)-registered disinfectants.[6] In the United States, the CDC recommends *C. auris* screening for patients admitted from facilities with known or suspected transmission, including those from long-term acute care or ventilator-capable nursing facilities.[7] Screening is also advised for patients with risk factors such as mechanical ventilation, indwelling devices (eg, central lines, tracheostomy tubes, urinary catheters), or prior colonization/infection with multidrug-resistant organisms (MDROs).

Methods for Detection and Antifungal Susceptibility Testing

Chromogenic media and biochemical panels can provide presumptive identification of *C. auris*; however, confirmatory testing with MALDI-TOF MS or molecular methods is essential, as chromogenic media and biochemical methods may misidentify *C. auris* as other yeasts (eg, *C. haemulonii* complex or *Rhodotorula*).[8-11] The Simplexa® *C. auris* Direct Kit is the first molecular assay that has US Food and Drug Administration (FDA) *de novo* authorization for assessment of *C. auris* colonization from skin swabs (axilla/groin). There are several FDA-approved multiplex polymerase chain reaction (PCR) assays available for direct detection of *C. auris* from positive blood cultures (see Table 1).

Key Insights into *Candida auris* from a Clinical Laboratory Perspective (*Continued*)

Table 1. Diagnostic Methods Used by Laboratories to Detect *C. auris*

Test	Manufacturer	Method	Identification Capability for *C. auris*	Specimen Types	Notes
Simplexa® *C. auris* Direct	Diasorin	Real-time PCR	Accurate detection	Skin swab	FDA *de novo* authorized assay
LIAISON PLEX® Yeast Blood Culture Assay	Diasorin	Multiplex PCR	Accurate detection	Positive blood culture broth	FDA-cleared
BIOFIRE® Blood Culture Identification 2 (BCID2) Panel	bioMérieux	Multiplex PCR	Accurate detection	Positive blood culture broth	FDA-cleared
cobas® eplex Blood Culture Identification Fungal Pathogen (BCID-FP) Panel	Roche Diagnostics	Multiplex PCR	Accurate detection	Positive blood culture broth	FDA-cleared
MALDI Biotyper®	Bruker Corporation	MALDI-TOF MS	Accurate detection	Yeast isolate	FDA-cleared
VITEK® MS	bioMérieux	MALDI-TOF MS	Accurate detection	Yeast isolate	FDA-cleared; earlier library versions may misidentify *C. auris* as closely related species like *C. haemulonii*[9]
PCR and sequencing (ITS/D1-D2 regions)	LDTs by various laboratories	Molecular sequencing	Accurate detection	Various	Reference method for species confirmation
API® 20 C AUX	bioMérieux	Biochemical panel	Not reliable	Yeast isolate	Often misidentifies *C. auris* as *Rhodotorula* or other yeasts
VITEK® 2 YST Card	bioMérieux	Automated biochemical ID	Presumptive identification	Yeast isolate	FDA-cleared; often misidentifies *C. auris* as *C. famata* or members of the *C. haemulonii* complex. Requires confirmatory testing with MALDI-TOF MS or molecular methods[10,11]
COLOREX™ Candida	CHROMagar™	Chromogenic media	Presumptive identification	Culture specimen	Requires confirmatory testing with MALDI-TOF MS or molecular methods
HardyCHROM™ Candida + auris	Hardy Diagnostics	Chromogenic media	Presumptive identification	Culture specimen	Requires confirmatory testing with MALDI-TOF MS or molecular methods

Abbreviations: FDA, US Food and Drug Administration; ID, identification; ITS, internal transcribed spacer; LDT, laboratory-developed test; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; PCR, polymerase chain reaction.

Key Insights into *Candida auris* from a Clinical Laboratory Perspective (*Continued*)

Echinocandins are preferred for therapy, yet resistance and treatment failures are increasingly reported.[12] Approximately 90% of isolates have fluconazole MICs of ≥ 32 µg/mL, around 15% to 35% exhibit elevated MICs to amphotericin B (≥ 2 µg/mL), and about 2% to 8% demonstrate elevated MICs to echinocandins (≥ 4 µg/mL).[3,12,13] In this patient case, micafungin was started empirically and the dosage subsequently escalated after *C. auris* was identified in blood. Susceptibility testing for *C. auris* can be performed using the reference broth microdilution method established by the Clinical and Laboratory Standards Institute (CLSI) Subcommittee on AFST.[14,15] Currently, there are no established clinical breakpoints for most antifungal agents against *C. auris*. Rezafungin, a novel echinocandin approved by the FDA in 2023 for the treatment of candidemia and invasive candidiasis, was recently assigned a susceptible-only clinical breakpoint of ≤ 0.5 µg/mL by the CLSI Subcommittee on AFST.[15] No intermediate or resistant categories have been defined. The CDC, however, has provided tentative MIC breakpoints (see Table 2) to guide interpretation of resistance for several other antifungal agents for *C. auris*.[16] Application of these criteria remains at the discretion of clinical microbiology laboratory directors, who should explicitly communicate their nonstandard status to providers when reporting results. Notably, as there are no FDA-recognized breakpoints for *C. auris*, no commercial antifungal susceptibility test (AFST) methods are FDA-cleared for testing *C. auris* isolates.

Table 2. CDC Tentative Antifungal MIC Breakpoints for *C. auris*[16]

Antifungal Agent	CDC Tentative MIC Breakpoints, µg/mL	Interpretative Category
Amphotericin B	≥ 2	Resistant
Anidulafungin	≥ 4	Resistant
Caspofungin	≥ 2	Resistant
Micafungin	≥ 4	Resistant
Rezafungin	N/A	-
Fluconazole[a]	≥ 32	Resistant

Abbreviations: CDC, Centers for Disease Control and Prevention; MIC, minimal inhibitory concentration; N/A, not available.
[a] Consider using fluconazole susceptibility as a surrogate for second-generation triazole susceptibility assessment. However, isolates that are resistant to fluconazole may respond to other triazoles occasionally. The decision to treat with another triazole will need to be made on a case-by-case basis.

Conclusion

This case underscores the critical need for rigorous screening protocols for *C. auris*, while highlighting the ongoing limitations of diagnostic methods of identification, commercial AFST platforms, and antifungal interpretive criteria. *C. auris* represents a serious public health threat requiring coordinated action from clinical microbiology laboratories and healthcare systems. In this patient, failure to screen for *C. auris* upon admission despite being transferred from a long-term care facility and being a known MDRO carrier delayed the detection of this important pathogen.

Laboratories must remain vigilant in detecting this emerging pathogen by implementing best-practice diagnostic methods. When in-house capabilities are limited, presumptively identified isolates should be sent to reference laboratories for species confirmation. AFST is warranted, at minimum, for isolates causing infection. These measures are crucial for early detection, accurate species identification, monitoring of resistance, and ultimately reducing transmission while improving patient outcomes.

References

1 Chakrabarti A, Sood P. On the emergence, spread and resistance of *Candida auris*: host, pathogen and environmental tipping points. *J Med Microbiol*. 2021;70(3):001318.

2 Centers for Disease Control and Prevention. Tracking *C. auris*. Accessed 23 October 2025. https://www.cdc.gov/candida-auris/tracking-c-auris/index.html.

3 Lyman M, Forsberg K, Sexton DJ, et al. Worsening spread of *Candida auris* in the United States, 2019 to 2021. *Ann Intern Med*. 2023;176(4):489-495.

4 Jeffery-Smith A, Taori SK, Schelenz S, et al. *Candida auris*: A review of the literature. *Clin Microbiol Rev*. 2017;31(1):e00029-17.

5 Schelenz S, Hagen F, Rhodes JL, et al. First hospital outbreak of the globally emerging *Candida auris* in a European hospital. *Antimicrob Resist Infect Control*. 2016;5:35.

Key Insights into *Candida auris* from a Clinical Laboratory Perspective (*Continued*)

6 United States Environmental Protection Agency. EPA's Registered Antimicrobial Products Effective Against *Candida auris* [List P]. Accessed 23 October 2025. https://www.epa.gov/pesticide-registration/epas-registered-antimicrobial-products-effective-against-candida-auris-list.

7 Centers for Disease Control and Prevention. Screening for *C. auris*. Accessed 23 October 2025. https://www.cdc.gov/candida-auris/screening/index.html.

8 CLSI. *Principles and Procedures for Detection and Culture of Fungi in Clinical Specimens.* 2nd ed. CLSI guideline M54. Clinical and Laboratory Standards Institute; 2021.

9 Dingle TC, Schwartz IS. The authors respond to "*Candida auris* can be identified accurately." *CMAJ*. 2020;192(2):E45.

10 Kathuria S, Singh PK, Sharma C, et al. Multidrug-resistant *Candida auris* misidentified as *Candida haemulonii*: Characterization by matrix-assisted laser desorption ionization-time of flight mass spectrometry and DNA sequencing and its antifungal susceptibility profile variability by Vitek 2, CLSI broth microdilution, and Etest method. *J Clin Microbiol.* 2015;53(6):1823-1830.

11 Ambaraghassi G, Dufresne PJ, Dufresne SF, et al. Identification of *Candida auris* by use of the updated Vitek 2 yeast identification system, version 8.01: A multilaboratory evaluation study. *J Clin Microbiol*. 2019;57(11):e00884-19.

12 Chowdhary A, Sharma C, Meis JF. *Candida auris*: A rapidly emerging cause of hospital-acquired multidrug-resistant fungal infections globally. *PLoS Pathog*. 2017;13(5):e1006290.

13 Lockhart SR, Etienne KA, Vallabhaneni S, et al. Simultaneous emergence of multidrug-resistant *Candida auris* on 3 continents confirmed by whole-genome sequencing and epidemiological analyses. *Clin Infect Dis.* 2017;64(2):134-140.

14 CLSI. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts.* 4th ed. CLSI standard M27. Clinical and Laboratory Standards Institute; 2017.

15 CLSI. *Performance Standards for Antifungal Susceptibility Testing of Yeasts.* CLSI supplement M27M44S. Clinical and Laboratory Standards Institute; 2022.

16 Centers for Disease Control and Prevention. Antifungal Susceptibility Testing for *C. auris*. Accessed 23 October 2025. https://www.cdc.gov/candida-auris/hcp/laboratories/antifungal-susceptibility-testing.html.

Practical Tips

A Focus on AST QC

Janet A. Hindler, Los Angeles County Department of Public Health, Los Angeles, CA

This article covers recent CLSI AST QC updates and 2 related QC topics.

Updated Guidance in CLSI M100 for QC Frequency and Selection of QC Strains

In the 35th edition of CLSI M100, there were significant changes to QC recommendations related to frequency of QC and selection of QC strains.[1] Here are a few things you need to know:

- Many anecdotal reports from leaders in the clinical microbiology community, together with a laboratory survey of bacterial AST performed by CLSI in 2023, demonstrated that QC failures are rarely due to a problem with the AST system [eg, faulty reagents (unless improperly stored), faulty equipment] but are instead random (irreproducible) or identifiable (eg, contamination, wrong strain selected, improper QC strain maintenance) and either easily corrected or are unlikely to significantly affect patient results.
- The Centers for Medicare & Medicaid Services (CMS) require daily QC for AST,[2] unless the laboratory has developed an individualized quality control plan (IQCP). An IQCP allows laboratories to reduce QC frequency from daily to a frequency determined by the laboratory. The frequency may be:
  - Weekly or less frequently
  - No less than that required in the AST manufacturer's instructions for use
  - No less than that required by the agency that accredits the laboratory
- The CLSI updated AST QC guidance is provided in Appendix I (35th edition of CLSI M100[1]) and is based on understanding:
  - The responsibilities of the AST manufacturer vs the laboratory.
  - The QC strains that are most likely to detect AST system problems.
  - The frequency and most common causes of out-of-range AST QC results.
  - Patient risks associated with AST and AST QC.
- Manufacturers of commercial AST systems perform extensive QC prior to release of each new lot/batch of AST reagents/media that includes testing additional QC strains beyond those suggested by CLSI.
- The user laboratory performs AST QC to ensure:
  - AST system performs comparably to the manufacturer's claims in their laboratory.
  - AST reagents/media maintained their integrity during shipping.
  - AST reagents/media maintained their integrity during their shelf life in the user's laboratory.
- CLSI no longer suggests weekly QC as the only alternative to daily AST QC, and specific QC strains are no longer in the "QC Recommendation" box for the individual organism groups in CLSI M100 Tables 2 (zone diameter and MIC breakpoints tables).[1] Appendix I Tables I1 to I4 provide examples for QC strain selection and frequency (35th edition of CLSI M100).[1]
- AST QC of a new lot/shipment in the user's laboratory may involve testing more QC strains than for subsequent AST QC (referred to as "routine QC").
- CLSI, together with the American Society for Microbiology and the College of American Pathologists have previously developed an IQCP template for AST QC. This template has been updated and will be posted as soon as all 3 organizations officially approve its content.

A Focus on AST QC (*Continued*)

QC Strain/Antimicrobial Agent Combinations Useful in Monitoring Mueller-Hinton Agar and Mueller-Hinton Broth Media Components

Although AST and media manufacturers have strict protocols to control media quality, it is helpful for users to understand how the QC strains might help identify specific problems related to Mueller-Hinton agar (MHA) or Mueller-Hinton broth (MHB). Differences in the components of MHA and MHB can be assessed by testing QC strains and antimicrobial agents whose activities are influenced by these components as shown in Table 1.

Table 1. Assessment of MHA and MHB Components[3]

QC Strains	Antimicrobial Agents	MHA and MHB Components Monitored by Testing Indicated QC Strain-Antimicrobial Agent Combination
Echerichia coli ATCC®[a] 25922	Tigecycline	Manganese
Pseudomonas aeruginosa ATCC® 27853	Gentamicin	pH, Ca^{++} / Mg^{++}
	Ciprofloxacin	pH, Mg^{++}
	Imipenem	Zn^{++}
Staphylococcus aureus ATCC® 29213	Erythromycin	pH
	Daptomycin	High Ca^{++}
	Oxacillin	NaCl
	Penicillin	pH
	Tetracycline	pH, Ca^{++} / Mg^{++}
E. faecalis ATCC® 29212 or *E. faecalis* ATCC® 33186	Trimethoprim-sulfamethoxazole	Thymidine

Abbreviations: ATCC, American Type Culture Collection; MHA, Mueller Hinton agar; MHB, Mueller Hinton broth; pH, negative logarithm of hydrogen ion concentration; QC, quality control.
[a] ATCC® is a registered trademark of the American Type Culture Collection.

On-Scale MIC End Points for QC Strains

MIC test systems generally include concentrations at 2-fold dilutions, and the numbers of concentrations tested may vary considerably depending on the antimicrobial agent and AST system under consideration. When performing MIC QC, it is helpful to test QC strains that have on-scale end points for the antimicrobial agent(s) being tested.

For example, when the following concentrations of antimicrobial agents (0.5, 1, 2, 4, 8, 16, and 32 µg/mL) are tested:

- MICs of 1, 2, 4, 8, 16, and 32 µg/mL are on scale
- MICs of ≤ 0.5 µg/mL (no growth in any tube) and >32 µg/mL (growth in all tubes) are off scale

Table 2 illustrates on- and off-scale MICs when testing 2 common QC strains against 3 different antimicrobial agents, each at concentrations of 0.5, 1, 2, 4, 8, 16, and 32 µg/mL.

Table 2. MIC QC Ranges[1]

Antimicrobial Agent	MIC QC Ranges, µg/mL	
	Escherichia coli ATCC® 25922[a]	*Pseudomonas aeruginosa* ATCC® 27853
Ertapenem	0.004–0.016	2–8[b]
Imipenem	0.06–0.25	1–4[b]
Meropenem	0.008–0.06	0.12–1[c]

Abbreviations: ATCC®, American Type Culture Collection; MIC, minimal inhibitory concentration; QC, quality control.
[a] No MICs for *E. coli* ATCC® 25922 are on scale.
[b] All MICs are on scale.
[c] An MIC of 1 µg/mL is on scale.

A Focus on AST QC (*Continued*)

If *E. coli* ATCC® 25922 was selected to QC a panel containing ertapenem at concentrations of 0.5 to 32 µg/mL, an MIC result of ≤ 0.5 µg/mL would be expected. As the acceptable ertapenem range for *E. coli* ATCC® 25922 is 0.004 to 0.016 µg/mL, this off-scale result of ≤ 0.5 µg/mL would not fully demonstrate whether ertapenem is in control. By contrast, testing *P. aeruginosa* ATCC® 27853 would be more informative, as acceptable MIC results are in the range of 2 to 8 µg/mL and would be on scale.

References

1. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 35th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2025.
2. Centers for Medicare & Medicaid Services, US Department of Health and Human Services. *Part 493—Laboratory Requirements; Standard: Bacteriology* (Codified at 42 CFR §493.1261). Office of the Federal Register; published annually.
3. ISO. *Clinical laboratory testing – Criteria for acceptable lots of dehydrated Mueller-Hinton agar and broth for antimicrobial susceptibility testing*. ISO/TS 16782. International Organization for Standardization; 2016.

Hot Topic #1

Major Updates to FDA Susceptibility Test Interpretive Criteria (STIC) Website

Romney M. Humphries, Vanderbilt University Medical Center, Nashville, TN

Globally, clinical laboratories rely on CLSI for guidance on antimicrobial susceptibility testing (AST) and interpretation. For laboratories in the United States, implementation of CLSI changes has been challenging since 2009, when the US Food and Drug Administration (FDA) began requiring use of FDA-recognized susceptibility test interpretive criteria (STIC), also known as breakpoints, in AST device 510(k) clearance submissions.[1] As such, device manufacturers have been unable to obtain FDA clearance for their devices using CLSI-updated breakpoints until these were recognized by FDA.

In late 2024, there were over 100 differences between FDA and CLSI breakpoints.[2] In January 2025, FDA released major updates to the STIC website, which included recognition of many CLSI breakpoints by FDA for the first time. Importantly, in addition to the 35th edition of CLSI M100,[3] FDA now also recognizes the methods and breakpoints published in the current editions of CLSI M45,[4] M24S,[5] M43,[6] M27M44S,[7] and M38M51S.[8] Many of the breakpoints that are newly recognized by FDA are for microorganisms for which no major clinical trial or pharmacokinetic/pharmacodynamic studies are likely to be conducted but that clinicians have treated for decades, often guided by using CLSI breakpoints on AST devices known as "legacy devices," which are those that received FDA clearance before 2009 and not subject to the 2009 FDA regulations. Even if some current breakpoints are imperfect, using them to obtain knowledge of the relative resistance of a bacterium is important in the management of patients, especially for those with serious infections.[9] Furthermore, data generated through routine AST of these uncommon organisms are useful for public health efforts to address emerging antimicrobial resistance. Recognition of CLSI AST standards by FDA provides a pathway for commercial manufacturers to develop ASTs for these pathogens.[10]

Important Notes for Navigating the Modified FDA STIC Website

- STIC now only lists exceptions or additions to CLSI breakpoints. Unless specifically listed on the STIC website, breakpoints published in the recognized CLSI documents can be safely assumed by laboratories to be recognized by the FDA.
- Laboratories and industry should pay strict attention to the edition of the CLSI standard recognized when evaluating the STIC website. There is generally a short lag between publication of a new CLSI standard or guideline and FDA's recognition of the document.
- The date that FDA recognizes a CLSI breakpoint is listed under the "Notice of Updates" on the STIC webpage.

To access the FDA STIC Website, click here.

Ongoing work between FDA and CLSI seeks to achieve recognition of high-priority CLSI breakpoints for which the FDA STIC website has no breakpoint (eg, staphylococcal breakpoints for trimethoprim-sulfamethoxazole or doxycycline). Many of these breakpoints are available on legacy AST devices. Nonetheless, significant work is required to ensure these agents that are often considered standard of care therapies can be tested on next-generation ASTs, and are maintained on current AST systems as the systems are updated.

References

1 Humphries RM, Ferraro MJ, Hindler JA. Impact of 21st Century Cures Act on breakpoints and commercial antimicrobial susceptibility test systems: Progress and pitfalls. *J Clin Microbiol.* 2018;56(5):e00139-18.

2 Wolfe KH, Pierce VM, Humphries RM. How new regulation of laboratory-developed antimicrobial susceptibility tests will affect infectious diseases clinical practice. *Clin Infect Dis.* 2024;78(5):1140-1147.

3 CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 35th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2025.

Major Updates to FDA Susceptibility Test Interpretive Criteria (STIC) Website (*Continued*)

4 CLSI. *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria.* 3rd ed. CLSI guideline M45. Clinical and Laboratory Standards Institute; 2016.

5 CLSI. *Performance Standards for Susceptibility Testing of Mycobacteria,* Nocardia *spp., and Other Aerobic Actinomycetes.* 2nd ed. CLSI supplement M24S. Clinical and Laboratory Standards Institute; 2023.

6 CLSI. *Methods for Antimicrobial Susceptibility Testing for Human Mycoplasmas; Approved Guideline.* CLSI document M43-A. Clinical and Laboratory Standards Institute; 2011.

7 CLSI. *Performance Standards for Antifungal Susceptibility Testing of Yeasts*. CLSI supplement M27M44S. Clinical and Laboratory Standards Institute; 2022.

8 CLSI. *Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi.* 3rd ed. CLSI supplement M38M51S. Clinical and Laboratory Standards Institute; 2022.

9 Jorgensen JH, Hindler JF. New consensus guidelines from the Clinical and Laboratory Standards Institute for antimicrobial susceptibility testing of infrequently isolated or fastidious bacteria. *Clin Infect Dis.* 2007;44(2):280-286.

10 Patel JB, Alby K, Humphries R, et al. Updating breakpoints in the United States: A summary from the ASM Clinical Microbiology Open 2022. *J Clin Microbiol*. 2023;61(10):e0115422.

Hot Topic #2

What is New With Anaerobes at CLSI?

Darcie Carpenter, IHMA Inc., Schaumburg, IL
Sarah Copsey-Mawer, UKARU, Public Health Wales, Cardiff, UK

There has been significant activity regarding anaerobes at CLSI over the last year. Much of this work has been behind the scenes, so this article will shed some light on these activities.

The Anaerobe Working Group (AnWG) has been interested in the efforts around anaerobe antimicrobial susceptibility testing (AST) being done in Europe, including the recent publications and updated European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidance involving disk diffusion testing.[1] The disk diffusion method allows AST results for several clinically relevant anaerobic species to be available to clinicians more rapidly using a less labor-intensive method than agar dilution.[2-6] The AnWG conducted a pilot evaluation of the EUCAST disk diffusion method with anaerobes. A set of 27 clinical isolates and 3 QC strains were shared between the organizations and testing was conducted at the 3 sites. Three antibiotics were evaluated, clindamycin, meropenem and metronidazole, by both disk diffusion and agar dilution. Although the data set was small, it generated results comparable with published data using these methods.[2,3] Categorical agreement for the EUCAST disk diffusion method compared with the CLSI agar dilution method was 96% for meropenem, 100% for metronidazole, and 93% for clindamycin. It is important to keep in mind that these data are based on only 27 isolates. The results were presented at the January 2025 CLSI meeting to the AST Subcommittee, which encouraged the AnWG to continue evaluation of this method. An enhanced dataset of QC for the disk diffusion method will be presented at the January 2026 meeting for evaluation and consideration, following feedback and guidance received in June 2025.

The AnWG has also completed work to update the CLSI M100 anaerobe antibiogram. This had previously been delayed due to insufficient agar dilution data being available to support an update. With support from the UK Anaerobe Reference Unit (UKARU) and the EUCAST Development Laboratory (EDL), data were collected and evaluated for updating the antibiogram. The updated antibiogram was presented at the June 2025 CLSI meeting for review and was approved for inclusion in the upcoming 36th edition of CLSI M100. Manuscripts for peer review publication are currently in preparation.

But wait! There is more! The current edition of CLSI M11, *Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria,* was published in October 2018.[7] It was decided to delay this update until the evaluation of the anaerobe disk diffusion testing method is complete to allow for this method to be added to the document, if approved by the CLSI AST Subcommittee.

And a Document Development Committee (DDC) on Detecting Anaerobes has been working on updates to CLSI M56, *Principles and Procedures for Detection of Anaerobes in Clinical Specimens*.[8] The DDC has been working on revisions to this document for some time and is currently finishing reviews of the final round of comments this past spring. The current schedule is to publish the next edition of the document in 2026.

In summary, there is significant work being done at CLSI regarding anaerobes. Much of this effort will be seen in 2026 as publications are released.

References

1 European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 15.0. 2025. Accessed 23 October 2025. https://www.eucast.org/clinical_breakpoints

2 Bavelaar H, Justesen US, Morris TE, et al. Development of a EUCAST disk diffusion method for the susceptibility testing of rapidly growing anaerobic bacteria using Fastidious Anaerobe Agar (FAA): A development study using *Bacteroides* species. *Clin Microbiol Infect*. 2021;27(11):1695.e1-1695.e6.

3 Matuschek E, Copsey-Mawer S, Petersson S, Åhman J, Morris TE, Kahlmeter G. The European committee on antimicrobial susceptibility testing disc diffusion susceptibility testing method for frequently isolated anaerobic bacteria. *Clin Microbiol Infect*. 2023;29(6):795.e1-795.e7

4 Justesen US, Åhman J, Matuschek E, Kahlmeter G. Assessing the quality of the anaerobic environment - a method developed to support EUCAST disk diffusion of anaerobic bacteria. *Eur J Clin Microbiol Infect Dis*. 2023;42(7):895-898.

What is New With Anaerobes at CLSI? (*Continued*)

5 Stubhaug TT, Giske CG, Justesen US, et al; Nordic *Bacteroides* AST Study Group. Antimicrobial susceptibility testing of Bacteroides species by disk diffusion: The NordicAST *Bacteroides* study. *Anaerobe*. 2023;81:102743.

6 Buhl MEJ, Sunnerhagen T, Join-Lambert O, et al; ReSuBacfrag Study Group; ESGAI. Antimicrobial resistance surveillance of *Bacteroides fragilis* isolated from blood cultures, Europe, 2022 (ReSuBacfrag). *Int J Antimicrob Agents*. 2024;64(3):107241.

7 CLSI. *Methods for Antimicrobial Susceptibility testing of Anaerobic Bacteria*. 9th Ed. CLSI Standard M11. Clinical and Laboratory Standards Institute, 2018.

8 CLSI. *Principles and Procedures for Detection of Anaerobes in Clinical Specimens; Approved Guideline*. CLSI document M56-A. Clinical and Laboratory Standards Institute, 2014.

Recent Developments #1

Disk Diffusion is Not a Reference Method

April M. Bobenchik, Penn State Hershey Medical Center, Hershey, PA

The 35th edition of CLSI M100 recognizes disk diffusion (DD) as a standard method but not a reference method.[1] A reference method is a standardized method that has been agreed upon by standards development organizations to serve as the method to which other methods are compared.[2] The CLSI, International Organization of Standardization (ISO), and European Committee for Antimicrobial Susceptibility Testing (EUCAST) all agree that broth microdilution (BMD) is a reference method for most nonfastidious and some fastidious organisms.

Agar dilution (AD) is the reference method for certain antimicrobial agents, such as fosfomycin, and for *Neisseria gonorrhoeae* and most anaerobes. Clinical breakpoints are first established using MIC data generated by BMD or AD, and then DD breakpoints are created by correlating zone diameters to that reference method MIC data. Because DD breakpoint determination always refers back to BMD or AD reference methods, it is most accurate to describe DD as a standard method but not a reference method. The current edition of CLSI M02[3] still lists DD as a reference method, but this will be updated in the next edition of CLSI M02.

For more information on how breakpoints are established, please check out the "Establishing MIC and Disk Diffusion Clinical Breakpoints" module of the "Using CLSI M100: Performance Standards for Antimicrobial Susceptibility Testing" course.[4] While categorizing DD as a standard method is a new development for 2025, it does not impact how laboratories use DD testing or report results from DD. Despite not being recognized as a reference method, DD can still be used a comparator method when performing in-house verifications of new AST devices or antimicrobial agents.[5] Laboratories should continue current practices but should be aware of the differences between "reference" vs "standard" AST methods.

References

1 CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 35th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2025.

2 Koeth LM and Miller LA. *Antimicrobial Susceptibility Test Methods: Dilution and Disk Diffusion Methods.* In: Carroll KC and Pfaller MA eds. Manual of Clinical Microbiology. 2019: 1420-1437.

3 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility.* 14th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2024.

4 CLSI. Using CLSI M100: Performance Standards for Antimicrobial Testing. Accessed 23 October 2025. https://clsi.org/shop/education/courses/m100-course/

5 CLSI. *Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems.* 1st ed. CLSI guideline M52. Clinical and Laboratory Standards Institute; 2015.

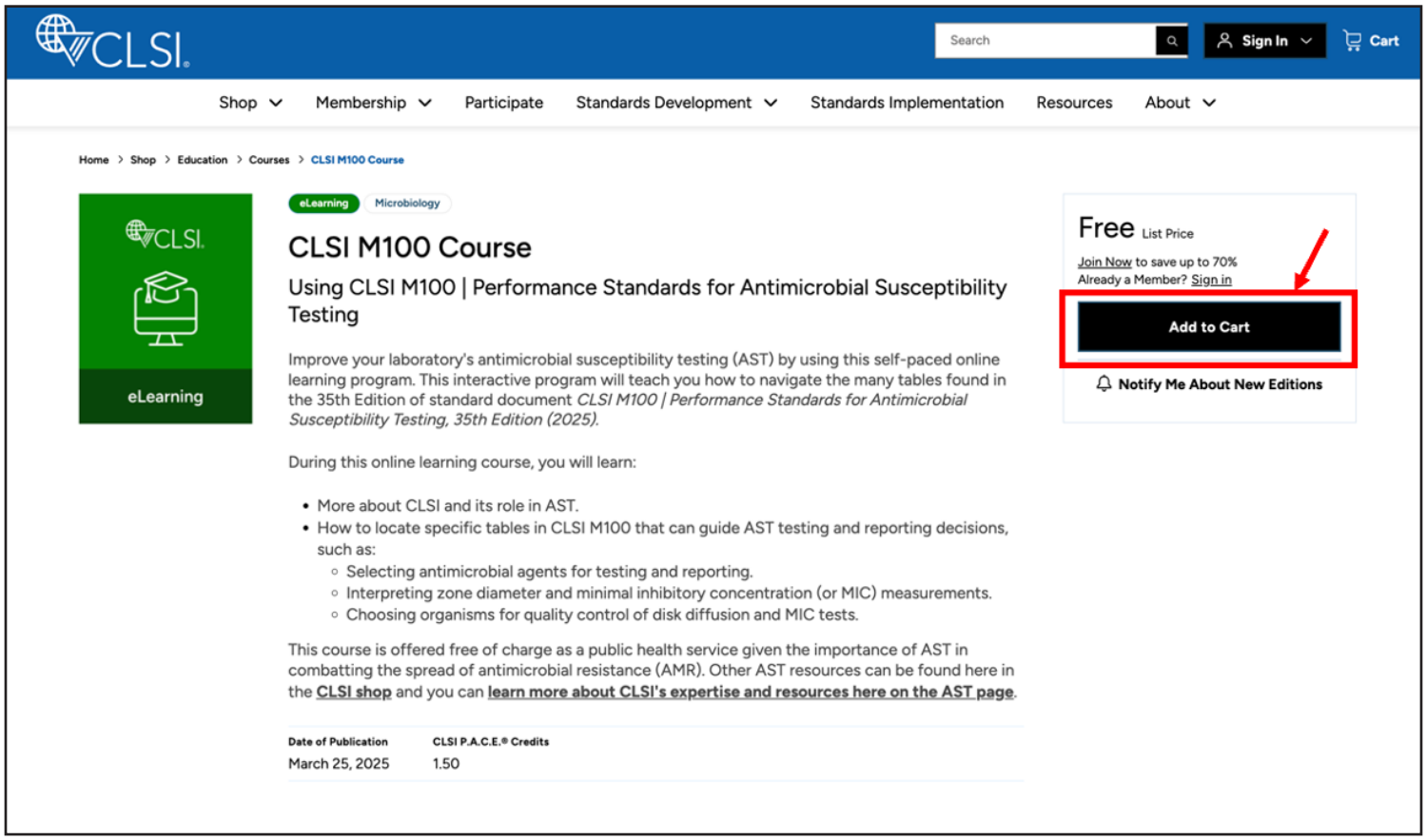
Recent Developments #2

What is the "CLSI M100 Course"?

Janet A. Hindler, Los Angeles County Department of Public Health, Los Angeles, CA

This self-paced, online, interactive learning program will teach you how to navigate the many tables found in the 35th edition of CLSI M100, *Performance Standards for Antimicrobial Susceptibility Testing*. The course is intended for those who have some familiarity with CLSI M100 and want to become more acquainted with locating specific tables and for students who are learning about standards for antimicrobial susceptibility testing.

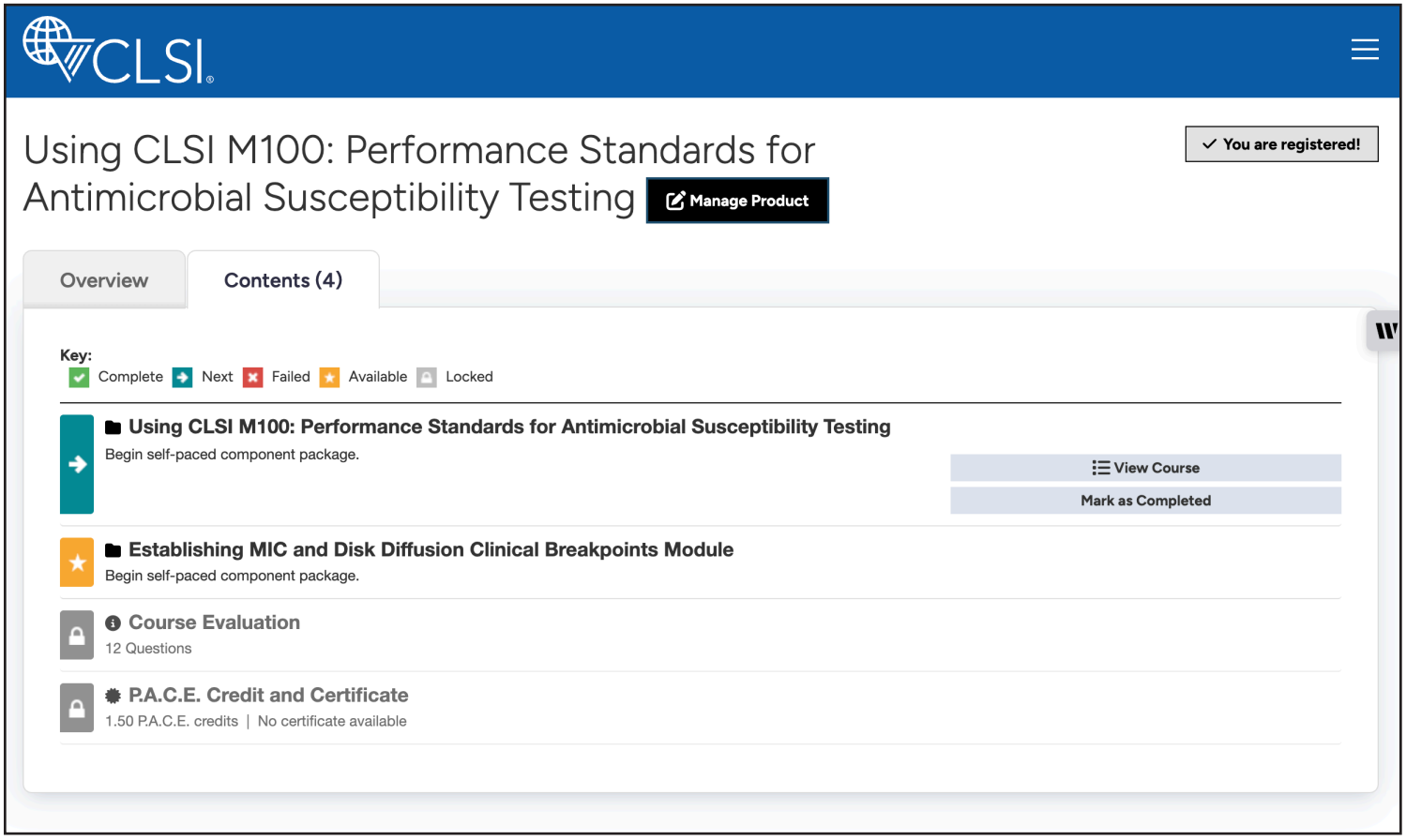
To access this free course,[2] please **click this link** to "add to cart." You will need to "check out" even though the course is free.



What is the "CLSI M100 Course"? (*Continued*)

Once you are in the CLSI M100 course (Using CLSI M100: *Performance Standards for Antimicrobial Susceptibility Testing*), you will see 4 options:

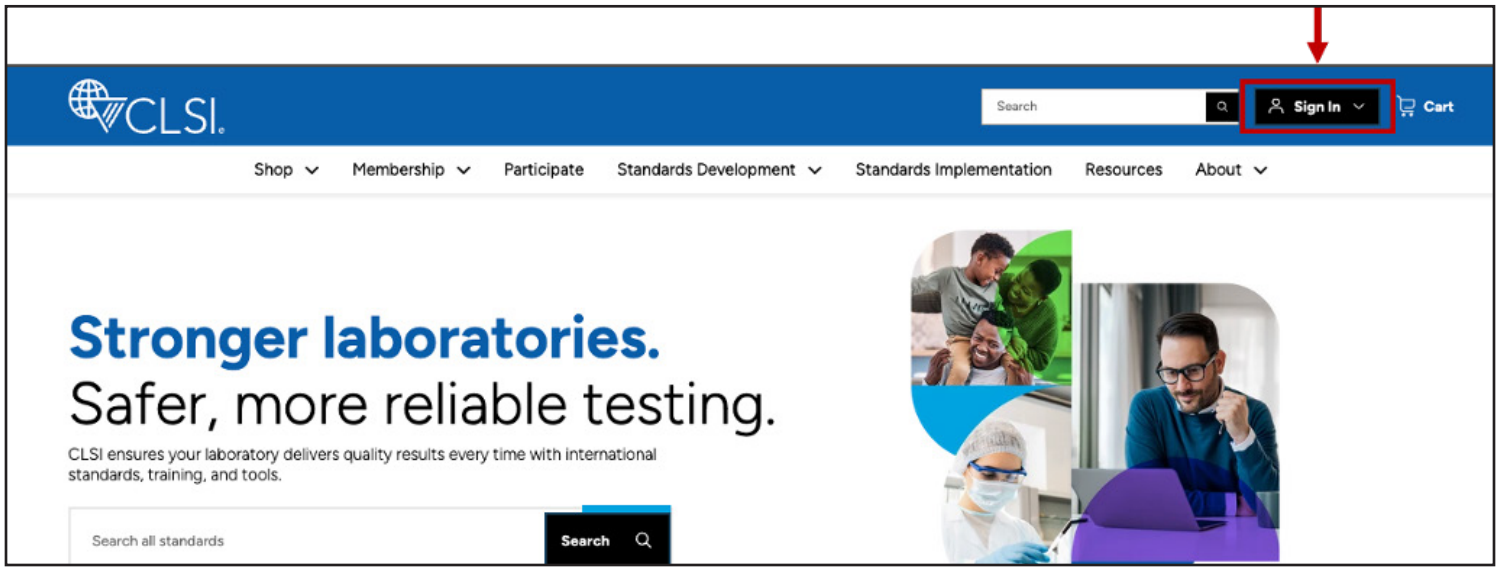
1. **Using CLSI M100: *Performance Standards for Antimicrobial Susceptibility Testing***[2] explains how to use the CLSI M100 document and includes an exercise on cascade reporting and selection of antimicrobial agents to test.
2. **Establishing MIC and Disk Diffusion Clinical Breakpoints Module** is a short program that provides a basic overview of the process used to set and revise breakpoints.
3. **Course Evaluation:** If you wish to receive 1.5 P.A.C.E.® credits, you must complete a course evaluation.
4. **P.A.C.E. Credit and Certificate:** You will receive an e-mail with your certificate within 1 day after completing the evaluation.



What is the "CLSI M100 Course"? (*Continued*)

After registering, you can return to access the CLSI M100 course[2] at any time from your "MyCLSI" dashboard.

1. Go to CLSI.org and select "Sign In" to access "MyCLSI Dashboard."



2. Select "MyCLSI Dashboard" from the "MyCLSI" drop-down menu and scroll down to "My Education" to view the course, or use the drop-down menu to view "My Education" where you can view and access all CLSI eLearning and webinars that you registered for over the past 3 years.