Modification of Antimicrobial Susceptibility Testing Methods

Written on behalf of the Clinical and Laboratory Standards Institute (CLSI) New Drug Alternative Methods Ad Hoc Working Group under the Subcommittee on Antimicrobial Susceptibility Testing in collaboration with the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Summary

The development of new antimicrobial agents is essential to combat antimicrobial resistance. Reliable antimicrobial susceptibility testing (AST) methods should be established early and thoughtfully to ensure timely patient access to these agents.

The standard reference method for AST is broth microdilution (BMD) in cation-adjusted Mueller-Hinton broth (CAMHB), as defined by CLSI M07 {1} and ISO 20776-1. {2} While some agents may require modifications to this method to better reflect clinical activity, such changes must be scientifically justified. Modifications aimed solely at producing lower minimal inhibitory concentration (MIC) values—or to make one antimicrobial agent appear superior to others—are not scientifically valid and are strongly discouraged.

CLSI and EUCAST caution developers that unnecessary deviations from reference AST methods can lead to increased costs, regulatory hurdles, delays in test availability, and reduced clinical adoption. Until joint guidance under development by CLSI and EUCAST is finalized, early and rigorous evaluation using the reference method is essential.

Background

In clinical practice, antimicrobial prescribing decisions rely heavily on *in vitro* AST results. In the early 2010s, several new antimicrobial agents were approved, but the lack of corresponding susceptibility tests at launch hindered their uptake. {3} Since then, substantial efforts have aligned AST development with regulatory approval timelines. Modifications to reference AST methods can jeopardize this alignment and should be considered well before the agent comes to market.

AST development should begin with the selection of a reproducible reference AST method that correlates with *in vivo* efficacy. This method supports the development of surrogate, commercially viable tests and serves as the basis for setting breakpoints and validating alternative methods. A suitable reference AST methods method must:

- Yield reproducible results within and between laboratories
- Detect meaningful differences in isolate responses to the agent
- Reflect susceptibility variation between strains
- Be minimally influenced by exogenous variables (eg, pH, ions, incubation conditions)

All these features require a high degree of standardization. Most microorganism/antimicrobial agent combinations can be tested following ISO 20776-1, {4} which describes the use of CAMHB and the BMD method. This is the default reference AST method approach. Alternative reference methods (described by CLSI and EUCAST) may be necessary for fastidious organisms requiring non-standard media or conditions. {5} {6}

Assess Reference AST Early in Antimicrobial Agent Development to Avoid Unnecessary Modification

Establishing a reliable AST method should begin early in antimicrobial agent development, even if clinical use is years away. Initial testing must follow the reference BMD method in CLSI M07 [1] and ISO 20776-1. [2] This early evaluation helps identify potential challenges.

It is a misconception that lower MICs inherently reflect superior efficacy. True clinical performance depends on pharmacokinetics/pharmacodynamics (PK/PD) and outcomes, not MICs alone. Altering methods to achieve artificially lower MICs misrepresents an antimicrobial agent's performance and may erode trust among regulators and clinicians, as well as delay routine AST in the clinical laboratory which will hinder the use of the new compound. CLSI and EUCAST leadership understand that there may be misconceptions about lower MIC values within the antimicrobial industry and clinical practice, and plan to expand educational efforts moving forward.

Modifications to standard methods should be rare, thoroughly justified, and guided by experts. If problems persist despite rigorous testing using the standard method, a modification may be considered. Signs of potential issues include:

- **Unreliable or irreproducible MICs,** possibly due to trailing endpoints, media interference, or antimicrobial agent instability in CAMHB
- **Unexpectedly broad or flat MIC distributions** in wild-type isolates, suggesting erratic antimicrobial agent activity and poor correlation with *in vivo* performance

In select cases, such as cefiderocol (ie, iron exclusion) or daptomycin (ie, calcium supplementation), carefully designed modifications improved *in vitro-in vivo* correlation. However, these examples are exceptions and should not act as precedents. Modifying reference AST methods can also create long-term barriers to test availability. For instance, the iron-depleted medium needed when testing cefiderocol delayed commercial test development and limited adoption despite US Food and Drug Administration (FDA) approval in 2019. [7]

To prevent such delays, antimicrobial developers should:

- Avoid unnecessary deviations from the reference method
- Identify potential need for reference AST method modification as early as possible in the antimicrobial agent's development process
- Ensure that method modifications can be implemented efficiently by commercial test developers and clinical laboratories

CLSI and EUCAST currently offer guidance, and more comprehensive joint recommendations are forthcoming. [5] [6]

Recommendations

Until joint CLSI and EUCAST guidance is finalized, developers are strongly advised to:

- **Evaluate new agents early using the reference BMD method**
- **Modify the method only after rigorous investigation** of possible causes of failure

- **Explore alternative standardized methods** (eg, those for fastidious organisms) before modifying the default BMD approach
- **Consult AST development experts** before pursuing modifications. Experts can help design technically sound methods and anticipate regulatory or commercial challenges
- **Use minimalist, targeted modifications**, such as removing a specific inhibitor or adding a stabilizing ion at low concentration, and rely on readily available reagents to limit financial and logistical burdens

References

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[3] Humphries RM, Hindler JA. Emerging Resistance, New Antimicrobial Agents … but No Tests! The Challenge of Antimicrobial Susceptibility Testing in the Current US Regulatory Landscape. (Clin Infect Dis. 2016;63(1):83-88).

[4] ISO. *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: Evaluation of performance of antimicrobial susceptibility test devices*. ISO 20776-2. International Organization for Standardization; 2007.

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