CLINICAL AND LABORATORY STANDARDS INSTITUTE®

6th Edition

M23

Development of *In Vitro* Susceptibility Test Methods, Breakpoints, and Quality Control Parameters

This guideline includes the necessary and recommended data for selecting appropriate breakpoints and quality control ranges for antimicrobial agents.

A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.

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Development of *In Vitro* Susceptibility Test Methods, Breakpoints, and Quality Control Parameters

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Abstract

Clinical and Laboratory Standards Institute guideline M23—*Development of* In Vitro *Susceptibility Test Methods, Breakpoints, and Quality Control Parameters* offers guidance for developing breakpoints and QC ranges for antimicrobial susceptibility tests against aerobic and anaerobic bacteria, as well as selected fungi, according to CLSI antimicrobial susceptibility testing documents. It describes the data used by the CLSI Subcommittees on Antimicrobial Susceptibility Testing and Antifungal Susceptibility Tests to establish these breakpoints and QC ranges for antimicrobial agents, including microbiological data, pharmacokinetic and pharmacodynamic characteristics, and clinical data. As additional experience is accrued with the individual antimicrobial agents, new data may be used to reassess breakpoints or QC ranges. Users of this guideline should understand that susceptibility test results cannot predict clinical outcomes with absolute certainty. They should be used along with best clinical judgment and laboratory support to best serve the patient.

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Foreword

CLSI develops standardized reference methods that measure the susceptibility of bacteria and fungi to antimicrobial agents *in vitro*. In this regard, the CLSI Subcommittee on Antimicrobial Susceptibility Testing (AST) is responsible for developing and updating the following CLSI susceptibility testing documents:

- M02—*Performance Standards for Antimicrobial Disk Susceptibility Tests*[1]
- M07—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*[2]
- M11—*Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria*[3]
- M45—*Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria*[4]
- M100—*Performance Standards for Antimicrobial Susceptibility Testing*[5] (supplement for CLSI documents M02,[1] M07,[2] and M11[3])

The CLSI Subcommittee on Antifungal Susceptibility Tests is responsible for developing and updating the following CLSI susceptibility testing documents:

- M27—*Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts*[6]
- M27M44S—*Performance Standards for Antifungal Susceptibility Testing of Yeasts*[7] (supplement for CLSI documents M27[6] and M44[8])
- M38—*Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi*[9]
- M38M51S—*Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi*[10] (supplement for CLSI documents M38[9] and M51[11])
- M44—*Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts*[8]
- M51—*Method for Antifungal Disk Diffusion Susceptibility Testing of Nondermatophyte Filamentous Fungi*[11]

M23 is a foundation guideline that supports these susceptibility testing documents. It also provides guidance on the data submitted by sponsors and the procedures followed by the CLSI Subcommittee on AST and the CLSI Subcommittee on Antifungal Susceptibility Tests to establish or revise QC ranges and susceptibility testing breakpoints for inclusion in CLSI documents. This guideline recognizes that submissions to CLSI may be made by a wide variety of organizations or individuals and that it is important to ensure the same processes are followed regardless of the data source. Nevertheless, M23 also recognizes that data provided to support new or revised breakpoints may vary significantly because of factors that include but are not limited to the age of the antimicrobial agent and whether the sponsor has access to raw data or only published data.

Essential Information

Content in this guideline marked with an asterisk (*) describes essential information required for review by the CLSI Subcommittee on AST. All chapters and subchapters without an asterisk describe additional information that may be supplied if available and that may be useful in supporting the selection of QC ranges and susceptibility testing breakpoints.

Overview of Changes

This guideline replaces the previous edition of the approved guideline, M23-Ed5, published in 2018. Several changes were made in this edition, including:

- Updating pharmacokinetic/pharmacodynamic (PK/PD)–related definitions (see Subchapter 1.3.1)
- Replacing Subchapter 2.3 (Developing Disks for Disk Diffusion Tests) with new Subchapter 2.2 (Assessing Variability of the Reference Method) to provide rationale for, factors to be assessed in, and suggested design of studies to assess reproducibility of reference broth microdilution and disk diffusion assays
  - Subchapter 2.2.2 cites CLSI document M23S[12] to provide guidance on evaluating and selecting appropriate content (potency) of antimicrobial agents for the development of disks for disk diffusion assays.
- Clarifying the importance of the four cutoffs used to establish clinically relevant breakpoints (see Chapter 5 and Table 6)
- Clarifying the methods and guidance on targets, number of isolates to test, etc. (see Subchapter 5.2)
  - Clarifying language for static experiments (see Subchapter 5.2.1.1)
  - Adding significant detail for dynamic experiments (eg, chemostat and hollow-fiber model studies) (see Subchapter 5.2.1.2)
  - Adding significant detail on *in vivo* systems (see Subchapter 5.2.2), including:
    - Study design
    - Need for high-quality pharmacokinetic (PK) data
    - Information on PK at infection sites (eg, epithelial lining fluid [ELF])
    - Mathematical modeling
    - Information on the numbers and types of isolates needed for studies
    - Information on the selection of relevant end points to determine PK/PD target
    - Information on assessment of pharmacodynamic index and PK/PD for efficacy of β-lactam combination agents
  - Including information on free-drug concentrations in ELF (see Subchapters 5.2.2 and 5.2.3)
  - For Monte Carlo simulations, adding a statement about inclusion of an assessment of PK/PD target variability and suggestions regarding the potential use of weighted mean or random assignment following a probability distribution (see Subchapter 5.2.4.1)
  - Providing a new table (see Table 7) and figure (see Figure 3) on how to present target attainment data (see Subchapter 5.2.4.2)
- Clarifying clinical exposure–response cutoff (see Subchapter 5.3)
- Adding text regarding presentation of outcomes, including discordance between clinical and microbiological outcomes, and evaluating minimal inhibitory concentration increases between baseline and postbaseline isolates (see Subchapter 5.4.2)
- Adding a sponsor checklist for CLSI breakpoint proposals (see Appendix A)
- Adding new Appendixes C (Example of a Reference Broth Microdilution Reproducibility Study) and D (Example of a Disk Diffusion Reproducibility Study), which describe the suggested design of studies to assess reproducibility of reference broth microdilution and disk diffusion assays, respectively

NOTE: The content of this guideline is supported by the CLSI consensus process and does not necessarily reflect the views of any single individual or organization.

KEY WORDS

antimicrobial agents

standard dilution methods for bacteria and fungi that grow aerobically

standard disk diffusion test

standard reference method for anaerobes

susceptibility testing

Chapter 1

Introduction

Development of *In Vitro* Susceptibility Test Methods, Breakpoints, and Quality Control Parameters

1 Introduction

1.1 Scope

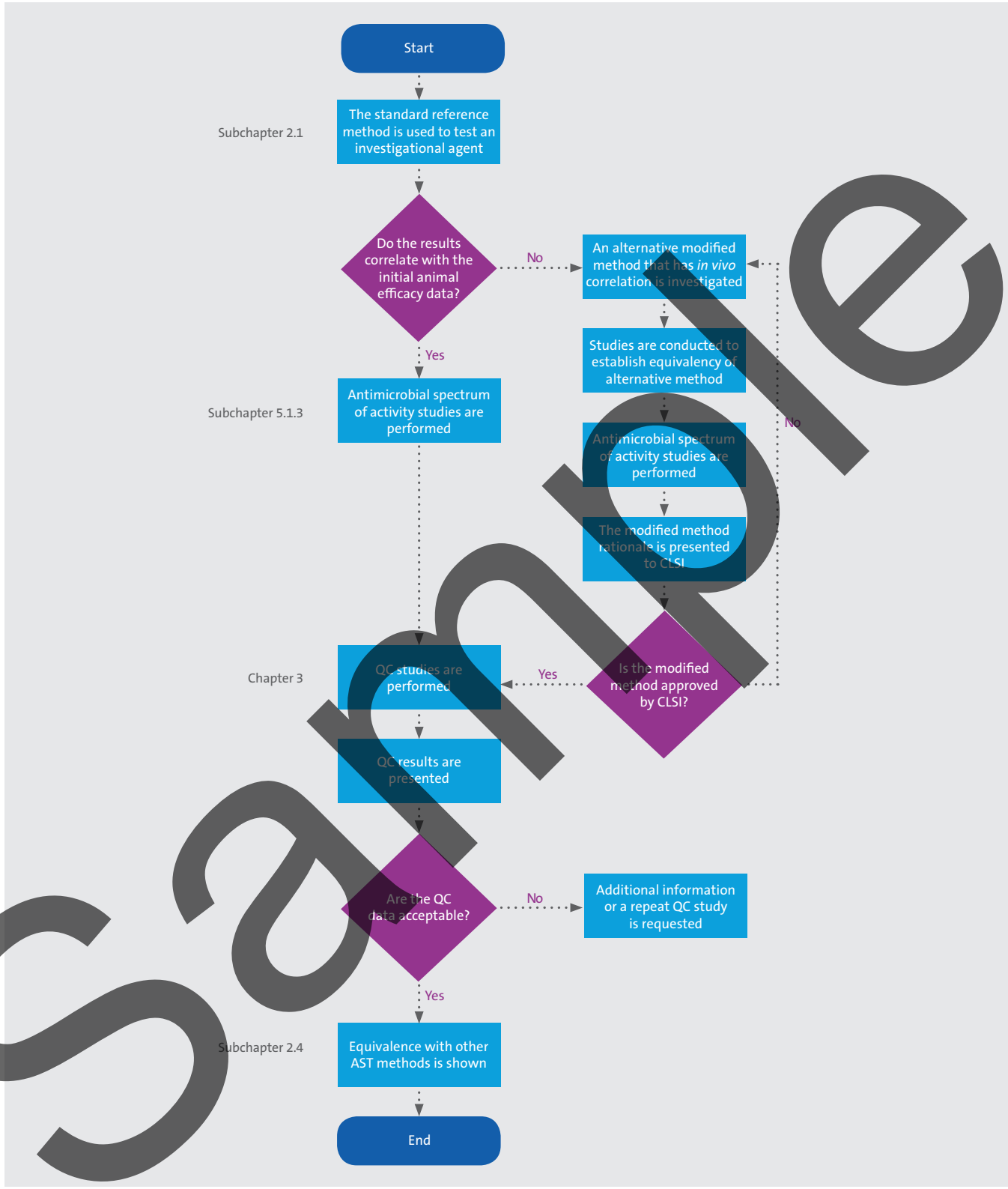
This guideline provides recommendations for determining breakpoints and QC parameters for antimicrobial agents that directly act on microorganisms. The intended audience includes sponsors (eg, antimicrobial agent manufacturers), CLSI volunteers, and various third-party groups planning to submit data to establish or revise QC ranges and antimicrobial susceptibility testing (AST) breakpoints and interpretive categories for inclusion in CLSI AST documents only. Appendix A provides a sponsor checklist for CLSI breakpoint proposals. The methods described do not apply to:

- Antimicrobial agents formulated for direct administration to skin or mucous membranes, topical or intraocular injection, intrathecal injection, or inhalation
- Antimicrobial agents that are intended to exert activity in the gut lumen

In some instances, recommendations may be based on limited data (eg, some species of nontuberculous mycobacteria). Also, CLSI recognizes that nontraditional agents (eg, those that can have a beneficial effect in disease but do not directly inhibit bacterial growth and survival, such as virulence inhibitors and/or non–small molecule agents) are being explored and developed for use in infectious diseases. However, at this time, there is no clear path for establishing breakpoints that are not based on minimal inhibitory concentrations (MICs).

1.2 Background

AST breakpoints, interpretive categories, and QC parameters are established by the CLSI Subcommittee on AST after review of available relevant data. This guideline describes the procedures to be followed by the CLSI Subcommittee on AST and by sponsors intending to submit data to facilitate timely review and decision-making processes. Data requirements to support setting new breakpoints and QC parameters and amendments to existing breakpoints are described. The CLSI Subcommittee on Antifungal Susceptibility Tests has developed standardized methods that enable laboratories to perform reliable and meaningful broth dilution and disk diffusion susceptibility testing of fungi (see CLSI documents M27,[6] M38,[9] M44,[8] and M51[11]). The process for determining breakpoints, interpretive categories, and QC ranges for antifungal agents is broadly the same as for the antibacterial agents. Thus, it may be assumed that the principles described in this guideline apply equally to antifungal agents. Subchapter 5.1 in this guideline describes the processes for establishing epidemiological cutoff values (ECVs). More expansive information regarding the use of ECVs for antifungal agents can be found in CLSI documents M57[13] and M57S.[14]



Abbreviations: AST, antimicrobial susceptibility testing; QC, quality control.

[a] Four basic symbols are used in this process flow chart: oval (signifies the beginning or end of a process), arrow (connects process activities), box (designates process activities), diamond (includes a question with alternative "Yes" and "No" responses).

Figure 1. Establishing a Reference Susceptibility Testing Method[a]

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