

M23

Development of *In Vitro* Susceptibility Testing Criteria and Quality Control Parameters

This guideline discusses 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 Testing Criteria and Quality Control Parameters

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Abstract

Clinical and Laboratory Standards Institute guideline M23—*Development of In Vitro Susceptibility Testing Criteria 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 standards. It describes the data used by the 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 antimicrobial agents are used in practice, additional experience accrued may be used to reassess breakpoints or QC ranges. Users of these guidelines 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 most effectively 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
- M45—*Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria*³
- M11—*Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria*⁴
- M100—*Performance Standards for Antimicrobial Susceptibility Testing*⁵ (supplement for M02,¹ M07,² and M11⁴)

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
- M44—*Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts*⁷
- M60—*Performance Standards for Antifungal Susceptibility Testing of Yeasts*⁸ (supplement for M27⁶ and M44⁷)
- M38—*Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi*⁹
- M51—*Method for Antifungal Disk Diffusion Susceptibility Testing of Nondermatophyte Filamentous Fungi*¹⁰
- M61—*Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi*¹¹ (supplement for M38⁹ and M51¹⁰)

M23 is an important foundation guideline that supports these susceptibility testing documents. M23's purpose is to provide guidance on the data submitted by sponsors and the procedures followed by the CLSI Subcommittee on AST to establish or revise QC ranges and susceptibility testing breakpoints for inclusion in CLSI documents. The process for determining breakpoints and QC ranges for antifungal agents is broadly the same as for the antibacterial agents, and the principles described in M23 also apply to antifungal agents.

This guideline recognizes that submissions 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, it also recognizes that the extent of the data that can be provided to support new or revised breakpoints may vary significantly due to 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, 4th ed., published in 2016. Several changes were made in this edition, including:

- Deleted Subchapter 4.1.3 on publication of breakpoints that are different from those approved by the US Food and Drug Administration
- Added a new subchapter (Subchapter 4.4) that describes a new process for periodically reviewing established breakpoints

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 that grow aerobically, standard disk diffusion test, standard reference method for anaerobes, susceptibility testing

Development of *In Vitro* Susceptibility Testing Criteria and Quality Control Parameters

Chapter 1: Introduction

This chapter includes:

- Guideline's scope and applicable exclusions
- Background information pertinent to the guideline's content
- "Note on Terminology" that highlights particular use and/or variation in use of terms and/or definitions
- Terms and definitions used in the guideline
- Abbreviations and acronyms used in the guideline

1.1 Scope

This guideline provides direction for determining breakpoints and QC parameters for antimicrobial agents that have a direct action on microorganisms. The intended audience includes sponsors (eg, antimicrobial agent manufacturers) planning to submit data to establish or revise QC ranges and susceptibility testing breakpoints and interpretive categories for inclusion in CLSI susceptibility testing documents. The methods described do not apply to:

- Slow-growing mycobacteria, for which specific guidance is available (see CLSI document M24¹²)
- Antimicrobial agents formulated for direct administration to skin or mucous membranes or for inhalation
- Antimicrobial agents that are intended to exert activity within the gut lumen

Guidance presented in M23 applies only to CLSI procedures and documents.

1.2 Background

Susceptibility testing breakpoints, interpretive categories, and QC parameters are established by the CLSI Subcommittee on Antimicrobial Susceptibility Testing (AST) after comprehensive review of all 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 AST has developed standardized methods that make it possible for laboratories to perform reliable and meaningful broth dilution and disk diffusion susceptibility testing of fungi (see CLSI documents M27,⁶ M38,⁹ M44,⁷ and M51¹⁰). 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. For this reason, the guideline refers to antimicrobial agents throughout. Where reference is made to the CLSI Subcommittee on AST, in most instances the same applies to the CLSI Subcommittee on Antifungal

Susceptibility Tests. A CLSI document on the criteria for developing and using epidemiological cutoff values (ECVs) for guiding clinical decisions when testing fungi against antifungal drug combinations for which there are no breakpoints has been published (see CLSI documents M57¹³ and M59¹⁴). The principles outlined in this guideline for establishing and revising ECVs apply to antibacterial agents as well.

1.3 Terminology

1.3.1 A Note on Terminology

CLSI, as a global leader in standardization, is firmly committed to achieving global harmonization whenever possible. Harmonization is a process of recognizing, understanding, and explaining differences while taking steps to achieve worldwide uniformity. CLSI recognizes that medical conventions in the global metrological community have evolved differently in different countries and regions and that legally required use of terms, regional usage, and different consensus timelines are all important considerations in the harmonization process. CLSI recognizes its important role in these efforts, and its consensus process focuses on harmonization of terms to facilitate the global application of standards and guidelines.

NOTE: Mandates are generally reserved for CLSI standards but are occasionally allowed in CLSI guidelines. In CLSI guidelines, use of the term “must” is either 1) based on a requirement or 2) indicative of a necessary step to ensure patient safety or proper fulfillment of a procedure. The subcommittee evaluated use of the term “must” and deemed it appropriate.

1.3.2 Definitions

breakpoint – minimal inhibitory concentration (MIC) or zone diameter value used to categorize an organism as susceptible, susceptible-dose dependent, intermediate, resistant, or nonsusceptible; **NOTE 1:** MIC or zone diameter values generated by a susceptibility test can be interpreted based upon established breakpoints; **NOTE 2:** See **interpretive category**.

clinical cutoff – a cutoff that is derived from any correlation there may be between clinical outcome and the minimal inhibitory concentrations of an antimicrobial agent(s) for the infecting pathogen(s).

clinical exposure-response (CER) cutoff – the highest minimal inhibitory concentration value at which efficacy would be predicted in patients based on CER relationships for efficacy in infected patients and on human pharmacokinetics.

epidemiological cutoff value (ECV) – the minimal inhibitory concentration (MIC) or zone diameter value that separates microbial populations into those with and without acquired and/or mutational resistance based on their phenotypes (wild-type or non-wild-type). The ECV defines the upper limit of susceptibility for the wild-type population of isolates.

EXAMPLE:

Interpretive Category	ECVs	
	MIC, µg/mL	Zone Diameter, mm
Wild-type	≤4	≥20
Non-wild-type	≥8	≤19

- **wild-type** – an ECV interpretive category defined by an ECV that describes isolates with no mechanisms of acquired resistance or reduced susceptibility for the antimicrobial agent being evaluated.

The Quality Management System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management system (QMS) approach in the development of standards and guidelines that facilitates project management, defines a document structure using a template, and provides a process to identify needed documents. The QMS approach applies a core set of “quality system essentials” (QSEs), basic to any organization, to all operations in any health care service’s path of workflow (ie, operational aspects that define how a particular product or service is provided). The QSEs provide the framework for delivery of any type of product or service, serving as a manager’s guide. The QSEs are:

Organization	Personnel	Process Management	Nonconforming Event Management
Customer Focus	Purchasing and Inventory	Documents and Records	Assessments
Facilities and Safety	Equipment	Information Management	Continual Improvement

M23 covers the QSE indicated by an “X.” For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section.

Organization	Customer Focus	Facilities and Safety	Personnel	Purchasing and Inventory	Equipment	Process Management	Documents and Records	Information Management	Nonconforming Event Management	Assessments	Continual Improvement
						X M02 M07 M11 M24 M26 M27 M38 M44 M45 M51 M57 M59 M60 M61 M100					

Path of Workflow

A path of workflow is the description of the necessary processes to deliver the particular product or service that the organization or entity provides. A laboratory path of workflow consists of the sequential processes: preexamination, examination, and postexamination and their respective sequential subprocesses. All laboratories follow these processes to deliver their services, namely quality laboratory information.

M23 does not cover any of the medical laboratory path of workflow processes. For a description of the documents listed in the grid, please refer to the Related CLSI Reference Materials section.

Preexamination				Examination			Postexamination	
Examination ordering	Sample collection	Sample transport	Sample receipt and processing	Examination	Results review and follow-up	Interpretation	Results reporting and archiving	Sample management
				M02 M07 M11 M24 M26 M27 M38 M44 M51	M02 M07 M11 M24 M26 M27 M38 M44 M45 M51 M59 M60 M100	M02 M07 M11 M24 M26 M27 M38 M44 M45 M51 M59 M60 M100	M02 M07 M11 M24 M27 M38 M44 M45 M51 M59 M60 M100	M27 M38 M51

Related CLSI Reference Materials*

- M02** **Performance Standards for Antimicrobial Disk Susceptibility Tests. 13th ed., 2018.** This standard covers the current recommended methods for disk susceptibility testing and criteria for quality control testing.
- M07** **Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. 11th ed., 2018.** This standard covers reference methods for determining minimal inhibitory concentrations of aerobic bacteria by broth macrodilution, broth microdilution, and agar dilution.
- M11** **Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria. 8th ed., 2012.** This standard provides reference methods for the determination of minimal inhibitory concentrations of anaerobic bacteria by agar dilution and broth microdilution.
- M24** **Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes. 2nd ed., 2011.** This standard provides protocols and related quality control parameters and interpretive criteria for the susceptibility testing of mycobacteria, *Nocardia* spp., and other aerobic actinomycetes.
- M26** **Methods for Determining Bactericidal Activity of Antimicrobial Agents. 1st ed., 1999.** This document provides procedures for determining the lethal activity of antimicrobial agents.
- M27** **Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts. 4th ed., 2017.** This standard covers antifungal agent selection and preparation, test procedure implementation and interpretation, and quality control requirements for susceptibility testing of yeasts that cause invasive fungal infections.
- M38** **Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi. 3rd ed., 2017.** This standard includes antifungal agent selection, preparation of antifungal stock solutions and dilutions for testing, test procedure implementation and interpretation, and quality control requirements for susceptibility testing of filamentous fungi (moulds) that cause invasive and cutaneous fungal infections.
- M44** **Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts. 2nd ed., 2009.** This document provides newly established methodology for disk diffusion testing of *Candida* spp., criteria for quality control testing, and interpretive criteria.
- M45** **Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria. 3rd ed., 2016.** This guideline informs clinical, public health, and research laboratories on susceptibility testing of infrequently isolated or fastidious bacteria that are not included in CLSI documents M02, M07, or M100. Antimicrobial agent selection, test interpretation, and quality control are addressed.
- M51** **Method for Antifungal Disk Diffusion Susceptibility Testing of Nondermatophyte Filamentous Fungi. 1st ed., 2010.** This document describes the guidelines for antifungal susceptibility testing by the disk diffusion method of nondermatophyte filamentous fungi (moulds) that cause invasive disease.
- M57** **Principles and Procedures for the Development of Epidemiological Cutoff Values for Antifungal Susceptibility Testing. 1st ed., 2016.** This guideline includes the criteria for developing and using epidemiological cutoff values for guiding clinical decisions when testing fungal species and antifungal agent combinations for which there are no breakpoints.
- M59** **Epidemiological Cutoff Values for Antifungal Susceptibility Testing. 2nd ed., 2018.** This document includes the epidemiological cutoff value and quality control tables developed according to criteria provided in the Clinical and Laboratory Standards Institute guideline M57.
- M60** **Performance Standards for Antifungal Susceptibility Testing of Yeasts. 1st ed., 2017.** This document includes updated minimal inhibitory concentration, zone diameter, and quality control tables for the Clinical and Laboratory Standards Institute antifungal susceptibility testing documents M27 and M44.
- M61** **Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi. 1st ed., 2017.** This document provides updated quality control tables for the Clinical and Laboratory Standards Institute antifungal susceptibility testing documents M38 and M51.

* CLSI documents are continually reviewed and revised through the CLSI consensus process; therefore, readers should refer to the most current editions.

Related CLSI Reference Materials (Continued)

M100 **Performance Standards for Antimicrobial Susceptibility Testing, 28th ed., 2018.** This document includes updated tables for the Clinical and Laboratory Standards Institute antimicrobial susceptibility testing standards M02, M07, and M11.

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