CLINICAL AND LABORATORY STANDARDS INSTITUTE®

1st Edition

M60

Performance Standards for Antifungal Susceptibility Testing of Yeasts

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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.

A CLSI supplement for global application.

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Performance Standards for Antifungal Susceptibility Testing of Yeasts

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Abstract

Clinical and Laboratory Standards Institute document M60—*Performance Standards for Antifungal Susceptibility Testing of Yeasts* includes the minimal inhibitory concentration, zone diameter, and QC tables developed following the standards described in CLSI documents M27[1] and M44.[2] The tabular information in this document is valid only when the methodology is followed as described in the current editions of CLSI documents M27[1] and M44.[2] Any previously published tables should be replaced with these new tables. Changes since the last edition appear in boldface type.

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Foreword

Users of CLSI documents M27[1] and M44[2] and this document should recognize that the standard methods described in CLSI documents are reference methods. These methods may be used for:

- Routine antifungal testing of patient isolates to guide therapy
- Evaluating commercial devices that will be used in medical laboratories
- Testing new agents or systems by drug or device manufacturers

Results generated by reference methods, such as those described in CLSI documents, may be used by regulatory authorities to evaluate commercial susceptibility testing device performance as part of the approval process. Regulatory clearance indicates that the commercial susceptibility testing device provides results that are substantially equivalent to those generated using reference methods for the organisms and antimicrobial agents described in the device manufacturer's approved package insert.

However, CLSI breakpoints may also differ from those approved by various regulatory organizations for many reasons, including:

- Database differences
- Data interpretation
- Dosage amounts used in different parts of the world
- Public health policies

Differences also exist because CLSI proactively evaluates the need for changing breakpoints. The reasons why breakpoints may change, and the manner in which CLSI evaluates data and determines breakpoints, are described in CLSI document M23.[3]

When CLSI decides to change an existing breakpoint, regulatory organizations may also review data to determine how changing breakpoints may affect antimicrobial agent safety and effectiveness for the approved indications. When a regulatory authority changes breakpoints, commercial device manufacturers may have to conduct a clinical trial, submit the data to the regulatory organization, and await review and approval. For these reasons, a delay of one or more years may be needed if a breakpoint change is to be implemented by a device manufacturer. Some regulatory and accreditation requirements allow laboratories using cleared or approved testing devices to use existing regulatory organization breakpoints. Either those or CLSI susceptibility breakpoints may be acceptable to laboratory accreditation organizations. Other regulatory and accreditation requirements may vary. Each laboratory should check with its antimicrobial susceptibility test system manufacturer for additional information on the breakpoints used in its system software. Laboratories should be aware of their specific regulatory and accreditation requirements for using CLSI breakpoints.

Once verified by the CLSI document development process, breakpoints may be implemented as soon as they are published in a supplement. However, medical laboratories should discuss this implementation with appropriate stakeholders, such as infectious disease practitioners and the pharmacy department, as well as the hospital pharmacy and therapeutics and infection control committees, before implementing newly approved or revised breakpoints. When a device includes antimicrobial test concentrations that are sufficient to interpret susceptibility and resistance to an agent using the CLSI breakpoints, a laboratory could, after appropriate validation as outlined in CLSI document M52,[4] choose to interpret and report results from that device using CLSI breakpoints.

NOTE: Current fungal taxonomy is under revision. Many genera have both a teleomorph (sexual state) and an anamorph (asexual state) name. In this document, the traditional *Candida* anamorph names are used to provide continuity to both past procedures and associated documents such as CLSI document M27.[1]

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Table 1. Minimal Inhibitory Concentration Breakpoints for *In Vitro* Broth Dilution Susceptibility Testing of *Candida* spp. and Select Antifungal Agents After 24-Hour Incubation

Antifungal Agent	Species	MIC Breakpoints and Interpretive Categories, µg/mL			
		S	I[*]	SDD[†]	R
Anidulafungin[1,‡]	*C. albicans*	≤0.25	0.5	–	≥1
	C. glabrata	≤0.12	0.25	–	≥0.5
	C. guilliermondii	≤2	4	–	≥8
	C. krusei	≤0.25	0.5	–	≥1
	C. parapsilosis	≤2	4	–	≥8
	C. tropicalis	≤0.25	0.5	–	≥1
Caspofungin[1,‡,§]	*C. albicans*	≤0.25	0.5	–	≥1
	C. glabrata	≤0.12	0.25	–	≥0.5
	C. guilliermondii	≤2	4	–	≥8
	C. krusei	≤0.25	0.5	–	≥1
	C. parapsilosis	≤2	4	–	≥8
	C. tropicalis	≤0.25	0.5	–	≥1
Micafungin[1,‡]	*C. albicans*	≤0.25	0.5	–	≥1
	C. glabrata	≤0.06	0.12	–	≥0.25
	C. guilliermondii	≤2	4	–	≥8
	C. krusei	≤0.25	0.5	–	≥1
	C. parapsilosis	≤2	4	–	≥8
	C. tropicalis	≤0.25	0.5	–	≥1
Voriconazole[2,‡,¶]	*C. albicans*	≤0.12	**0.25–0.5**	–	≥1
	C. glabrata[#]	–	–	–	–
	C. krusei	≤0.5	**1**	–	≥2
	C. parapsilosis	≤0.12	**0.25–0.5**	–	≥1
	C. tropicalis	≤0.12	**0.25–0.5**	–	≥1
Fluconazole[3,†]	*C. albicans*	≤2	–	4	≥8
	C. glabrata[**]	–	–	≤32	≥64
	C. krusei[††]	–	–	–	–
	C. parapsilosis	≤2	–	4	≥8
	C. tropicalis	≤2	–	4	≥8

[*] **The "I" category provides a buffer zone for antimicrobial susceptibility testing that is necessary to avoid major and very major errors that may occur given the inherent variability of the *in vitro* testing method. Available data do not permit isolates with MIC results in the "I" range to be clearly categorized as either "S" or "R." Strains with "I" MICs may respond clinically to a higher-than-standard dose of drug or in situations in which drug penetration is maximized.**

[†] Susceptibility depends on achieving the maximum possible blood level. For fluconazole, doses higher than the standard dosing amount (6 mg/kg/day) may be needed in adults with normal renal function and body habitus.

[‡] For these antifungal agents, the data are based substantially on experience with non-neutropenic patients with candidemia; their clinical relevance in other settings is uncertain.

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Table 1. (Continued)

[§] **Caspofungin susceptibility testing *in vitro* has been associated with significant interlaboratory variability, contributing to reports of false resistance when using the reference method described in CLSI document M27.[4,5] The cause of the variability is unclear. When testing caspofungin, susceptible results may be reported as "susceptible"; however, laboratories should confirm "I" or "R" results by a) additional susceptibility testing with micafungin[6] or anidulafungin,[7] b) DNA sequence analysis of *FKS* genes to identify resistance hot spot mutations in *FKS1* (all *Candida* spp.) and *FKS2* (*C. glabrata* only),[8,9] or c) sending to a referral laboratory for confirmation. *Candida* spp. resistant to anidulafungin or micafungin, or possessing characteristic *FKS* hot spot mutations are considered resistant to all echinocandins, including caspofungin, and should be reported as such.[6,7]**

[¶] Breakpoints may also be used for 48-hour readings if 24-hour growth control shows insufficient growth.

[#] For *C. glabrata* and voriconazole, current data are insufficient to demonstrate a correlation between *in vitro* susceptibility testing and clinical outcome.

[**] For fluconazole, these guidelines are based on extensive experience with mucosal and invasive infections due to *Candida* spp. When an isolate is identified as *C. glabrata* and the MIC is ≤32 µg/mL, it should be determined whether fluconazole is appropriate in the specific clinical context. If so, patients should receive a maximum dosage regimen of fluconazole. Expert consultation on selecting a maximum dosage regimen may be useful.

[††] **Isolates of *C. krusei* are assumed to be intrinsically resistant to fluconazole, so their MICs should not be interpreted using this scale.**

Abbreviations: DNA, deoxyribonucleic acid; I, intermediate; MIC, minimal inhibitory concentration; R, resistant; S, susceptible; SDD, susceptible-dose dependent.

NOTE 1: Information in boldface type is new or modified since the previous edition.

NOTE 2: The selected breakpoints have been established to distinguish resistant mutants from susceptible isolates, and differences in breakpoints reflect methodological issues. Due to *in vitro* methodological issues, the breakpoint for micafungin against *C. glabrata* is lower than that of other echinocandins, which does not reflect any inherent clinical differences in efficacy. True differences in antifungal activity among the echinocandins are rare.[10]

NOTE 3: The MIC breakpoints (µg/mL) for *Candida* spp. are shown against the indicated agents. If MICs are measured using a scale yielding results that fall between the categories, the next highest category is implied. Thus, an isolate for which the fluconazole MIC equals 3 µg/mL would be placed in the SDD category.

NOTE 4: Previous breakpoints for itraconazole and flucytosine were established with minimal clinical data; emerging data now suggest the previous breakpoints were not correct and should not be used. For *Candida* spp. and itraconazole, epidemiological cutoff values that define the limit of the wild-type distribution are established and may be useful for distinguishing between wild-type and non-wild-type isolates (those with intrinsic or acquired known resistance mechanisms) (see CLSI documents M57[11] and M59[12]).

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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

M60 covers the QSE indicated by an "X." For a description of the 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 M23 M27 M44 M52 M57 M59					

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.

M60 covers the medical laboratory path of workflow processes indicated by an "X." For a description of the other 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
				M27	X M27 M44 M59	X M27 M44 M59	X M27 M44 M59	M27

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Related CLSI Reference Materials*

M23 **Development of *In Vitro* Susceptibility Testing Criteria and Quality Control Parameters. 4th ed., 2016.** This guideline discusses the necessary and recommended data for the selection of appropriate interpretive criteria and quality control ranges for 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.

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.

M52 **Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems. 1st ed., 2015.** This guideline includes recommendations for verification of commercial US Food and Drug Administration–cleared microbial identification and antimicrobial susceptibility testing systems by clinical laboratory professionals to fulfill regulatory or quality assurance requirements for the use of these systems for diagnostic testing.

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. 1st ed., 2016.** 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.

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