CLINICAL AND LABORATORY STANDARDS INSTITUTE®

3rd Edition

M27M44S

Performance Standards for Antifungal Susceptibility Testing of Yeasts

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

Gary W. Procop, MD, MS
Philippe J. Dufresne, PhD, RMCCM
Elizabeth Berkow, PhD
Sharon K. Cullen, BS, RAC
Tanis Dingle, PhD, D(ABMM), FCCM
Jeff Fuller, PhD, FCCM, D(ABMM)
Kimberly E. Hanson, MD, MHS
Nicole M. Holliday, BA
Audrey N. Schuetz, MD, MPH, D(ABMM)
Paul E. Verweij, MD, FECMM
Nathan P. Wiederhold, PharmD
Adrian M. Zelazny, PhD, D(ABMM)

Abstract

Clinical and Laboratory Standards Institute document M27M44S—*Performance Standards for Antifungal Susceptibility Testing of Yeasts* includes minimal inhibitory concentration, zone diameter, and quality control tables developed following the guidance in CLSI documents M27[1] and M44.[2] The data in the tables are valid only when the methodologies in CLSI documents M27[1] and M44[2] are followed. Users should replace previously published tables with these new tables. Changes in the tables since the previous edition was published appear in boldface type.

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Foreword

The breakpoints and interpretive categories provided in this document are generated using the reference methods for antifungal susceptibility testing of yeasts described in CLSI documents M27[1] and M44.[2] These reference methods may be used for:

- Routine antifungal testing of patient isolates to guide therapy
- Evaluation of commercial devices that will be used in medical laboratories
- Testing of 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 commercial device 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 differ from breakpoints 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 that breakpoints may change, as well as 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 review data to determine how the changes 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, there might be a delay of one or more years if a device manufacturer decides to implement a breakpoint change. Some regulatory and accreditation requirements permit laboratories using cleared or approved testing devices to use existing regulatory organization breakpoints. Either the regulatory approved breakpoints or CLSI breakpoints may be acceptable to laboratory accreditation organizations**, depending on the method used for susceptibility testing.** Other regulatory and accreditation requirements vary. Each laboratory should consult its 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.

Following discussions with appropriate stakeholders (eg, infectious diseases practitioners and pharmacy practitioners, the hospital's pharmacy and therapeutics and infection prevention committees), laboratories may **verify and** implement newly approved or revised CLSI breakpoints as soon as they are published. Some devices might specify antimicrobial test concentrations that are sufficient to interpret susceptibility and resistance to an agent using the CLSI breakpoints. In such cases, after appropriate validation as outlined in CLSI document M52,[4] a laboratory could 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 with both past procedures and associated documents such as CLSI document M27[1] and others.[5-7]

NOTE: When serial twofold dilution MICs are being prepared and tested, the actual dilution scheme is, eg, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.0078125, 0.0039063, 0.0019531 µg/mL, etc. For convenience only, and not because these are the actual concentrations tested, it was decided to use the following values in M27M44S: 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.12, 0.06, 0.03, 0.016, 0.008, 0.004, 0.002 µg/mL, etc. The values that appear in the tables are equivalent to the actual values tested, eg, 0.12 µg/mL = 0.125 µg/mL, and laboratories should report an MIC of ≤ 0.125 µg/mL as ≤ 0.12 µg/mL.

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

General Comments

(1) If the 24-hour growth control is insufficient, breakpoints may also be used for 48-hour readings.

(2) The intermediate category provides a buffer zone for antifungal 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 intermediate range to be clearly categorized as either "susceptible" or "resistant." Strains with intermediate MICs might respond clinically to a higher-than-standard dose of a drug or in situations in which drug penetration is maximized.

(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 susceptible-dose dependent category.

(4) Per CLSI document M38M51S,[1] previous breakpoints for itraconazole and flucytosine were established with minimal clinical data. Emerging data now suggest that the previous breakpoints were not correct and should not be used. For *Candida* spp. and itraconazole, ECVs 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 acquired known resistance mechanisms) (see CLSI documents M57[2] and M57S[3]).

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

Antifungal Agent	Species	MIC Breakpoints and Interpretive Categories, µg/mL			
		S	I	SDD	R
Anidulafungin[4,a]	*C. albicans*	≤ 0.25	0.5	-	≥ 1
	C. glabrata[b]	≤ 0.12	0.25	-	≥ 0.5
	C. guilliermondii[b]	≤ 2	4	-	≥ 8
	C. krusei[b]	≤ 0.25	0.5	-	≥ 1
	C. parapsilosis[c]	≤ 2	4	-	≥ 8
	C. tropicalis	≤ 0.25	0.5	-	≥ 1
Caspofungin[4,a,d]	*C. albicans*	≤ 0.25	0.5	-	≥ 1
	C. glabrata	≤ 0.12	0.25	-	≥ 0.5
	C. guilliermondii[b]	≤ 2	4	-	≥ 8
	C. krusei[b]	≤ 0.25	0.5	-	≥ 1
	C. parapsilosis[c]	≤ 2	4	-	≥ 8
	C. tropicalis	≤ 0.25	0.5	-	≥ 1

Table 1. (Continued)

Antifungal Agent	Species	MIC Breakpoints and Interpretive Categories, µg/mL			
		S	I	SDD	R
Fluconazole[5,e,f]	*C. albicans*	≤ 2	-	4	≥ 8
	C. glabrata[b]	-	-	≤ 32	≥ 64
	C. krusei[b,g]	-	-	-	-
	C. parapsilosis[c]	≤ 2	-	4	≥ 8
	C. tropicalis	≤ 2	-	4	≥ 8
Micafungin[4,a]	*C. albicans*	≤ 0.25	0.5	-	≥ 1
	C. glabrata[b,h]	≤ 0.06	0.12	-	≥ 0.25
	C. guilliermondii[b]	≤ 2	4	-	≥ 8
	C. krusei[b]	≤ 0.25	0.5	-	≥ 1
	C. parapsilosis[c]	≤ 2	4	-	≥ 8
	C. tropicalis	≤ 0.25	0.5	-	≥ 1
Rezafungin[i,j]	***C. albicans***	**≤ 0.25**	**-**	**-**	**-**
	C. auris	**≤ 0.5**	**-**	**-**	**-**
	C. dubliniensis	**≤ 0.12**	**-**	**-**	**-**
	C. glabrata	**≤ 0.5**	**-**	**-**	**-**
	C. krusei[b]	**≤ 0.25**	**-**	**-**	**-**
	C. parapsilosis[c]	**≤ 2**	**-**	**-**	**-**
	C. tropicalis	**≤ 0.25**	**-**	**-**	**-**
Voriconazole[6,a]	*C. albicans*	≤ 0.12	0.25-0.5	-	≥ 1
	C. glabrata[b,k]	-	-	-	-
	C. krusei[b]	≤ 0.5	1	-	≥ 2
	C. parapsilosis[c]	≤ 0.12	0.25-0.5	-	≥ 1
	C. tropicalis	≤ 0.12	0.25-0.5	-	≥ 1

Abbreviations: ECV, epidemiological cutoff value; I, intermediate; MIC, minimal inhibitory concentration; R, resistant; S, susceptible; SDD, susceptible-dose dependent.

Footnotes

a. For these antifungal agents, the data are based largely on experience with non-neutropenic patients with candidemia. The clinical relevance of the antifungal agents in other settings is uncertain.

b. **These *Candida* spp. are also recognized under the following alternate taxonomic names:**
- ***C. glabrata*: *Nakaseomyces glabrata***
- ***C. guilliermondii*: *Meyerozyma guilliermondii***
- ***C. krusei*: *Pichia kudriavzevii***

c. When no further species determination has been performed, ***C. parapsilosis* breakpoints may be applied in areas where** the prevalence of the cryptic species (*C. orthopsilosis* or *C. metapsilosis*) is low **(eg, North America)**.[7-11] However, if further species determination identifies one of the cryptic species within the complex, *C. parapsilosis* breakpoints should not be applied. Instead, it should be indicated on the laboratory report that no breakpoints exist for interpretation and that use of ECVs should be considered (see CLSI document M57S[3]).

d. Caspofungin susceptibility testing *in vitro* has been associated with significant interlaboratory variability, contributing to reports of false resistance when the reference method described in CLSI document M27[12] is used.[13] The cause of the variability is unclear. When caspofungin is tested, susceptible results may be reported as "susceptible." However, laboratories should confirm "intermediate" or "resistant" results with one of the following options:

Appendix A. Body Site Reporting for *Candida* spp.[1,2]

The table below provides guidelines for reporting antifungal agents and reporting options when *Candida* spp. susceptibility is tested in specific body sites. Guidelines are also provided for body sites from which certain antifungals would not be appropriate to report.

Antifungal Agent	Specimen	Reporting	Comment	Rationale
Amphotericin B[3]	Urine[4]	No reporting restrictions	Lipid formulations of amphotericin B do not achieve adequate urine concentrations and should not be used to treat UTIs.	Small percentages of amphotericin B lipid formulations are recovered in the urine after systemic administration, compared with high recovery of amphotericin B deoxycholate.
5-FC		No reporting restrictions	5-FC should not be used as monotherapy for severe *Candida* infections because resistance can develop rapidly. It should be used rarely in neonates.[5,6]	
Azoles	CNS (brain tissue, abscess material)[7-11]; CSF	Routinely report only fluconazole and voriconazole. Report itraconazole, posaconazole, and isavuconazole only by request.		Report by request and suppress results for itraconazole, posaconazole, and isavuconazole because clinical data are limited.
	Ocular (cornea, aqueous, vitreous)[12-15]	Routinely report only fluconazole and voriconazole. Report itraconazole, posaconazole, and isavuconazole only by request.		Report by request and suppress results for itraconazole, posaconazole, and isavuconazole because clinical data are limited.
	Urine[4]	If testing azoles, report only fluconazole.		Other azoles could be reported by request because these agents may penetrate kidney tissue.

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