CLINICAL AND LABORATORY STANDARDS INSTITUTE®

4th Edition

CLSI M27M44S™

Performance Standards for Antifungal Susceptibility Testing of Yeasts

CLSI M27M44S includes updated minimal inhibitory concentration, zone diameter, and quality control tables for the Clinical and Laboratory Standards Institute antifungal susceptibility testing documents CLSI M27 and CLSI M44.

A CLSI supplement for global application.

CLSI M27M44S-Ed4
March 2026
Replaces CLSI M27M44S-Ed3

Performance Standards for Antifungal Susceptibility Testing of Yeasts

Philippe J. Dufresne, PhD
Nathan P. Wiederhold, PharmD
Barbara D. Alexander, MD, MHS
David Andes, MD
Andrew Borman, BSc, PhD
Ryan Demkowicz, MD
Tanis Dingle, PhD, D(ABMM), FCCM
Hari Dwivedi, BVSc(DVM), MVSC, PhD
Julianne Kus, HonBSc, MSc, PhD, FCCM
Shawn R. Lockhart, PhD, D(ABMM), F(AAM)
Stephanie Mitchell, PhD, D(ABMM)
Chris Pillar, PhD
Amir Seyedmousavi, VMD, PhD, D(ABMM), FECMM
Paul E. Verweij, MD, FECMM
Zoe Freeman Weiss, MD
Sean Zhang, MD, PhD, D(ABMM)

Abstract

CLSI 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 M27[1] and CLSI M44.[2] The data in the tables are valid only when the methodologies in CLSI M27[1] and CLSI 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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Contents

Foreword

The breakpoints and interpretive categories provided in CLSI M27M44S are generated using **CLSI M27[1] broth dilution** reference **and CLSI M44[2] disk diffusion standardized** methods for antifungal susceptibility testing of yeasts. These 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 **CLSI M27[1] or other** 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 results generated using reference methods for the organisms and antimicrobial agents described in the device manufacturer's approved package insert. However, CLSI breakpoints might differ from breakpoints approved by various regulatory organizations for many reasons, including:

- Differences in databases **and minimal inhibitory concentration (MIC) data collected**
- **Variations in** data interpretation
- **Geographic variability in** dosage **regimens**
- Public health policies

Differences also **arise** because CLSI **regularly assesses** the need **to update** breakpoints. CLSI M23[3] **outlines the rationale for** breakpoint changes **and the** data evaluation **process used to determine them.**

When CLSI decides to change an existing breakpoint, regulatory organizations may **assess** how **breakpoint** changes affect antimicrobial agent safety and **efficacy** for approved **uses.** When a regulatory authority changes breakpoints, commercial device manufacturers might have to conduct clinical trials, submit the data **for** regulatory **review,** and await approval. **This process can result in** delays of 1 or more years **before** breakpoint changes **are** implemented **by** device manufacturers. Some regulatory and accreditation requirements **allow** laboratories **to continue** using **the** existing breakpoints **provided by** cleared or approved testing devices. 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 **must also ensure compliance with** their specific regulatory and accreditation requirements **when** using CLSI breakpoints.

After 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 **verification** as outlined in CLSI M52,[4] a laboratory could choose to interpret and report results from that device using CLSI breakpoints.

NOTE 1: Current fungal taxonomy is under revision. Many genera have **2 names**: teleomorph (sexual state) and anamorph (asexual state) **(see CLSI M64[5]).[6]** In CLSI M27M44S, the traditional *Candida* anamorph names are used to provide continuity with both past procedures and associated documents such as CLSI M27,[1] **CLSI M64,[5]** and others.[7-10] **Justification is outlined in CLSI M64.[5]**

NOTE 2: When serial 2-fold 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 CLSI 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.

NOTE 3: Information in boldface type is new or modified since the previous edition.

Overview of Changes

CLSI M27M44S-Ed4 replaces CLSI M27M44S-Ed3, published in 2022. Several changes were made in this edition, including:

Section/Table	Action	Change to:	Reason/Specific Change
Foreword	Revised	Foreword	• Clarifying that breakpoints and interpretive categories are generated using the reference and standardized methods • Clarifying the rationale for breakpoint changes • Clarifying that laboratories must ensure compliance with regulatory and accreditation requirements • Clarifying that CLSI M52[4] provides guidance on verification of new or revised breakpoints
		NOTE	Supporting justification for using anamorph names for fungal organisms
	Added	References	Supporting justification for using anamorph names for fungal organisms
Table 1. Minimal Inhibitory Concentration Breakpoints for *In Vitro* Broth Dilution Susceptibility Testing of *Candida* spp. and Select Antifungal Agents After 24-Hour Incubation	Revised	General	Comment clarifying that MICs falling between categories will be rounded to the next highest category
		Footnotes	• Revising alternate taxonomic name for *Candida glabrata* • Clarifying that DNA sequencing of the *FKS* genes is not currently widely available • Clarifying that caspofungin can exhibit false resistance when the CLSI M27[1] reference method is used • Clarifying statement regarding IR for *Candida krusei*
	Added	General	Comment describing guidance for body site reporting in Appendix A
		Fluconazole IR designation	*C. krusei*
		Footnotes	• Including alternate taxonomic name for *Candida auris* • Clarifying statement regarding echinocandin resistance for *Candida* spp.
		References	Regarding rationale determination of rezafungin breakpoints
	Deleted	Footnote	Regarding tentative rezafungin MIC breakpoints
		Reference	CLSI M38M51S,[11] which is no longer applicable to Table 1

Section/Table	Action	Change to:	Reason/Specific Change
Table 3. Recommended 24-Hour Minimal Inhibitory Concentration Limits for Quality Control Strains for Broth Microdilution Procedures	Revised	General	Comment regarding MIC reading instructions
	Added	Footnote	Clarifying origin of MIC percentages
		Reference	CLSI M23[3]
Table 5. Zone Diameter and Equivalent Minimal Inhibitory Concentration Breakpoints for Select Antifungal Agents Against *Candida* spp. After 24-Hour Incubation	Revised	Footnote	Regarding *C. krusei* and fluconazole MIC testing
	Added	Footnote	Regarding certain *Candida* spp. and alternate taxonomic names
Appendix B. Intrinsic Resistance for Yeasts	Revised	General	Defining IR and the rationale for not performing susceptibility testing
		Footnote	Including alternate taxonomic names for *C. krusei* and *Candida lusitaniae*
	Added	Footnote	Including recognition of names for some *Rhodotorula* spp and some *Trichosporon* spp.
Glossary. Antifungal Agent Abbreviations, Routes of Administration, and Drug Class	Added	New antifungal agent	Ibrexafungerp
		Drug class	Manogepix

Abbreviations: DNA, deoxyribonucleic acid; IR, intrinsic resistance; MIC, minimal inhibitory concentration.

KEY WORDS

antifungal agent
azole
breakpoint
broth dilution
echinocandin
interpretive category
minimal inhibitory concentration
quality control
susceptibility testing
yeasts
zone diameter

NOTE: The content of CLSI M27M44S is supported by the CLSI consensus process and does not necessarily reflect the views of any single individual or organization.

Abbreviations and Acronyms

ATCC®[a] American Type Culture Collection
DMSO dimethyl sulfoxide
DNA deoxyribonucleic acid
ECV epidemiological cutoff value
I intermediate
IR intrinsic resistance

MIC minimal inhibitory concentration

QC quality control

R resistant

S susceptible

SDD susceptible-dose dependent

Footnote

a. ATCC® is a registered trademark of the American Type Culture Collection.

References

1 CLSI. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts*. 4th ed. CLSI standard M27. Clinical and Laboratory Standards Institute; 2017.

2 CLSI. *Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts*. 3rd ed. CLSI guideline M44. Clinical and Laboratory Standards Institute; 2018.

3 CLSI. *Development of* In Vitro *Susceptibility Test Methods, Breakpoints, and Quality Control Parameters*. 6th ed. CLSI guideline M23. Clinical and Laboratory Standards Institute; 2023.

4 CLSI. *Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems*. 1st ed. CLSI guideline M52. Clinical and Laboratory Standards Institute; 2015.

5 CLSI. *Implementation of Taxonomy Nomenclature Changes*. 1st ed. CLSI guideline M64. Clinical and Laboratory Standards Institute; 2024.

6 de Hoog S, Walsh TJ, Ahmed SA, et al. A conceptual framework for nomenclatural stability and validity of medically important fungi: a proposed global consensus guideline for fungal name changes supported by ABP, ASM, CLSI, ECMM, ESCMID-EFISG, EUCAST-AFST, FDLC, IDSA, ISHAM, MMSA, and MSGERC. *J Clin Microbiol.* 2023;61(11):e0087323. doi:10.1128/jcm.00873-23

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10 Borman AM, Johnson EM. Name changes for fungi of medical importance, 2020 to 2021. *J Clin Microbiol.* 2023;61(6):e0033022. doi:10.1128/jcm.00330-22

11 CLSI. *Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi*. 4th ed. CLSI supplement M38M51S. Clinical and Laboratory Standards Institute; 2026.

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

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 might occur, given the inherent variability of the *in vitro* testing method. Available data do not enable 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. **For example,** a ***Candida albicans*** isolate for which the fluconazole MIC equals 3 µg/mL would **be rounded to an MIC of 4 µg/mL and** be placed in the susceptible-dose dependent category.

(4) Previous breakpoints for itraconazole and flucytosine were established with minimal clinical data. **Current** 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 might be useful for distinguishing between wild-type and non-wild-type isolates (those with acquired known resistance mechanisms) (see CLSI M57[1] and CLSI M57S[2]).

(5) Appendix A provides guidance on reporting antifungal agents and on reporting options when *Candida* spp. susceptibility is tested in specific body sites. Guidance is also provided for body sites from which certain antifungal agents would not be appropriate to report.

Antifungal Agent	Species	MIC Breakpoints and Interpretive Categories, µg/mL			
		S	I	SDD	R
Anidulafungin[3,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[3,a,d]	*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
Fluconazole[4,e,f]	*C. albicans*	≤ 2	–	4	≥ 8
	C. glabrata[b]	–	–	≤ 32	≥ 64
	C. krusei[b,g]	IR			
	C. parapsilosis[c]	≤ 2	–	4	≥ 8
	C. tropicalis	≤ 2	–	4	≥ 8

Table 1. (Continued)

Antifungal Agent	Species	MIC Breakpoints and Interpretive Categories, µg/mL			
		S	I	SDD	R
Micafungin[3,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[5-14,i]	*C. albicans*	≤ 0.25	–	–	–
	C. auris[b]	≤ 0.5	–	–	–
	C. dubliniensis	≤ 0.12	–	–	–
	C. glabrata[b]	≤ 0.5	–	–	–
	C. krusei[b]	≤ 0.25	–	–	–
	C. parapsilosis[c]	≤ 2	–	–	–
	C. tropicalis	≤ 0.25	–	–	–
Voriconazole[15,a]	*C. albicans*	≤ 0.12	0.25-0.5	–	≥ 1
	C. glabrata[b,j]	–	–	–	–
	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: DNA, deoxyribonucleic acid; ECV, epidemiological cutoff value; I, intermediate; **IR, intrinsic resistance;** 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. auris: Candidozyma auris***
- *C. glabrata: Nakaseomyces* ***glabratus***
- *C. guilliermondii: Meyerozyma guilliermondii*
- *C. krusei: Pichia kudriavzevii*

c. When no further species determination **can be** 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).[16-20] However, if further species determination identifies 1 of the cryptic species within the complex, *C. parapsilosis* breakpoints should not be applied. Instead, the laboratory report should indicate that no breakpoints exist for interpretation and that use of ECVs should be considered (see CLSI M57S[2]).

d. Caspofungin susceptibility testing *in vitro* has **shown** significant interlaboratory variability, **leading to reports of likely false resistance** when the reference method described in CLSI M27[21] is used.[22] The **exact** cause of the variability is unclear. When caspofungin is tested, susceptible results can be reported as "susceptible." However, laboratories should confirm "intermediate" or "resistant" results with 1 of the following options:

- **Perform** additional susceptibility testing with micafungin[23] or anidulafungin.[24]

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