CLINICAL AND LABORATORY STANDARDS INSTITUTE®

2nd Edition

M60

Performance Standards for Antifungal Susceptibility Testing of Yeasts

This document provides 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.

M60-Ed2
June 2020
Replaces M60-Ed1

Performance Standards for Antifungal Susceptibility Testing of Yeasts

Gary W. Procop, MD, MS
Philippe J. Dufresne, PhD, RMCCM
Elizabeth Berkow, PhD
Jeff Fuller, PhD, FCCM, D(ABMM)
Kimberly E. Hanson, MD, MHS
Nicole M. Holliday, BA
David H. Pincus, MS, RM/SM(NRCM), SM(ASCP)
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 M60—*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 appear in boldface type.

Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antifungal Susceptibility Testing of Yeasts.* 2nd ed. CLSI supplement M60 (ISBN 978-1-68440-082-9 [Print]; ISBN 978-1-68440-083-6 [Electronic]). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2020.

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

Suggested Citation

CLSI. *Performance Standards for Antifungal Susceptibility Testing of Yeasts.* 2nd ed. CLSI supplement M60. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.

Previous Editions:
M27-S4: May 2004, April 2005, April 2008, December 2012
M44-S3: January 2006, August 2007, August 2009
M60-Ed1: November 2017

M60-Ed2
ISBN 978-1-68440-082-9 (Print)
ISBN 978-1-68440-083-6 (Electronic)
ISSN 1558-6502 (Print)
ISSN 2162-2914 (Electronic) Volume 40, Number 8

Contents

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 also 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, a delay of one or more years may be needed 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. 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 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]

Overview of Changes

This document replaces the previous edition of the approved document, M60-Ed1, published in 2017. Several changes were made in this edition, including:

- **Table 1. Minimal Inhibitory Concentration Breakpoints for *In Vitro* Broth Dilution Susceptibility Testing of *Candida* spp. and Select Antifungal Agents After 24-Hour Incubation:**
  - Added footnote and references regarding recommendations for interpreting *Candida parapsilosis* breakpoints
  - Revised footnote regarding intrinsic resistance of *Candida krusei* to fluconazole
- **Table 2. Solvents and Diluents for Preparing Stock Antifungal Agent Solutions for Broth Dilution Testing:**
  - Added solvent and diluent information for:
    - Ibrexafungerp
    - Manogepix
    - Rezafungin
- **Table 3. (formerly Table 4) Recommended 24-Hour Minimal Inhibitory Concentration Limits for Quality Control Strains for Broth Microdilution Procedures:**

  NOTE 1: In the previous edition of M60, Table 3 contained 48-hour QC ranges, and Table 4 contained 24-hour QC ranges. In this edition, the tables have been transposed.

  NOTE 2: The minimal inhibitory concentration (MIC) QC ranges for ibrexafungerp were adopted by the Subcommittee on Antifungal Susceptibility Tests during the annual meetings in January 2019 and January 2020. These QC ranges are tentative and are open for comment for one year from the publication of M60.

  - Added MIC QC ranges for:
    - Ibrexafungerp
      - *C. krusei* ATCC® 6258
      - *C. parapsilosis* ATCC® 22019
    - Manogepix
      - *Candida albicans* ATCC® 90028
      - *C. parapsilosis* ATCC® 22019
    - Rezafungin
      - *C. krusei* ATCC® 6258
      - *C. parapsilosis* ATCC® 22019
  - Revised NOTE regarding MICs
  - ***Deleted*** NOTE regarding tentative 24-hour MIC QC ranges
- **Table 5. Zone Diameter and Equivalent Minimal Inhibitory Concentration Breakpoints for Select Antifungal Agents Against *Candida* spp. After 24-Hour Incubation:**
  - Revised footnote regarding intrinsic resistance of *Candida krusei* to fluconazole
  - ***Deleted*** footnotes regarding tentative zone diameter interpretive categories

- **Table 6. Recommended Quality Control Zone Diameter (mm) Ranges After 24-Hour Incubation:**

  NOTE: The QC zone diameter ranges were adopted by the Subcommittee on Antifungal Susceptibility Tests during the annual meetings in January 2019 and January 2020. These zone diameter QC ranges are tentative and are open for comment for one year from the publication of M60.

  – Added disk diffusion QC ranges for:
    - Manogepix
      - *C. albicans* ATCC® 90028
      - *C. parapsilosis* ATCC® 22019
      - *Candida tropicalis* ATCC® 750
    - Rezafungin
      - *C. albicans* ATCC® 90028
      - *C. krusei* ATCC® 6258
      - *C. parapsilosis* ATCC® 22019
      - *C. tropicalis* ATCC® 750

  – ***Deleted*** footnote regarding tentative zone diameter QC ranges

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

Key Words

Antifungal agent, azole, breakpoint, broth dilution, disk diffusion, echinocandin, interpretive category, minimal inhibitory concentration, quality control, susceptibility testing, yeasts, zone diameter

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[a]	Species	MIC Breakpoints and Interpretive Categories, µg/mL			
		S	I[b]	SDD[c]	R
Anidulafungin[1,d]	*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[e]	≤2	4	–	≥8
	C. tropicalis	≤0.25	0.5	–	≥1
Caspofungin[1,d,f]	*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[e]	≤2	4	–	≥8
	C. tropicalis	≤0.25	0.5	–	≥1
Fluconazole[2,c]	*C. albicans*	≤2	–	4	≥8
	C. glabrata[g]	–	–	≤32	≥64
	C. krusei[h]	–	–	–	–
	C. parapsilosis[e]	≤2	–	4	≥8
	C. tropicalis	≤2	–	4	≥8
Micafungin[1,d]	*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[e]	≤2	4	–	≥8
	C. tropicalis	≤0.25	0.5	–	≥1
Voriconazole[3,d]	*C. albicans*	≤0.12	0.25–0.5	–	≥1
	C. glabrata[i]	–	–	–	–
	C. krusei	≤0.5	1	–	≥2
	C. parapsilosis[e]	≤0.12	0.25–0.5	–	≥1
	C. tropicalis	≤0.12	0.25–0.5	–	≥1

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

Footnotes

a. Breakpoints may also be used for 48-hour readings if the 24-hour growth control shows insufficient growth.

b. The intermediate 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 minimal inhibitory concentration (MIC) results in the intermediate range to be clearly categorized as either "susceptible" or "resistant." Strains with intermediate MICs may respond clinically to a higher-than-standard dose of drug or in situations in which drug penetration is maximized.

c. 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.

d. 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.

Table 1. (Continued)

e. For *C. parapsilosis* complex, when no further species determination has been performed, because the prevalence of the cryptic species (*Candida orthopsilosis* or *Candida metapsilosis*) is low, *C. parapsilosis* breakpoints may be applied.[4-8] 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 use of epidemiological cutoff values (ECVs) should be considered (see CLSI document M59[9]).

f. 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[10] is used.[11] 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:

- Additional susceptibility testing with micafungin[12] or anidulafungin[13]
- DNA sequence analysis of *FKS* genes to identify resistance hot-spot mutations in *FKS1* (all *Candida* spp.) and *FKS2* (*C. glabrata* only)[14,15]
- Sending the isolate to a referral laboratory for confirmation

Candida spp. that are resistant to anidulafungin or micafungin or that possess characteristic *FKS* hot-spot mutations are considered resistant to all echinocandins, including caspofungin, and should be reported as such.[12,13]

g. For fluconazole, these breakpoints 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, the clinician should determine whether fluconazole is appropriate in the specific clinical context. If so, patients should receive the maximum dosage regimen of fluconazole. Expert consultation on selecting a maximum dosage regimen may be useful.

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

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

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 variants from susceptible isolates. Differences in breakpoints reflect methodological issues. Owing 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.[16]

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 "susceptible-dose dependent" category.

NOTE 4: Per CLSI document M61,[17] 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[18] and M59[9]).

References for Table 1

1 Pfaller MA, Diekema DJ, Andes D, et al.; CLSI Subcommittee for Antifungal Testing. Clinical breakpoints for the echinocandins and *Candida* revisited: integration of molecular, clinical, and microbiological data to arrive at species-specific interpretive criteria. *Drug Resist Updat.* 2011;14(3):164-176.

Table 2. Solvents and Diluents for Preparing Stock Antifungal Agent Solutions for Broth Dilution Testing

Antifungal Agent	Solvent[a,b] (Full-Strength and Intermediate Solutions)	Diluent (Final Concentration)
Amphotericin B	DMSO	Medium
Anidulafungin	DMSO	Medium
Caspofungin	DMSO	Medium
Fluconazole	DMSO	Medium
Flucytosine	DMSO	Medium
Ibrexafungerp	**DMSO**	**Medium**
Isavuconazole	DMSO	Medium
Itraconazole	DMSO	Medium
Ketoconazole	DMSO	Medium
Manogepix	**DMSO**	**Medium**
Micafungin	DMSO	Medium
Posaconazole	DMSO	Medium
Rezafungin	**DMSO**	**Medium**
Voriconazole	DMSO	Medium

Abbreviation: DMSO, dimethyl sulfoxide.

Footnotes

a. Dimethyl sulfoxide (DMSO) can be toxic and also enables other drugs to be absorbed through the skin. Before DMSO is used, the DMSO safety data sheet should be consulted.

b. The laboratory should follow the manufacturer's recommendations when selecting a solvent.

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

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

950 West Valley Road, Suite 2500, Wayne, PA 19087 USA

P: +1.610.688.0100 **Toll Free (US):** 877.447.1888 **F:** +1.610.688.0700

E: customerservice@clsi.org **www.clsi.org**

PRINT ISBN 978-1-68440-082-9

ELECTRONIC ISBN 978-1-68440-083-6

M60-Ed2