CLINICAL AND LABORATORY STANDARDS INSTITUTE®

3rd Edition

M59

Epidemiological Cutoff Values for Antifungal Susceptibility Testing

This document provides epidemiological cutoff values developed according to the criteria in the Clinical and Laboratory Standards Institute (CLSI) guideline M57 and generated according to the reference broth dilution methods described in the CLSI standards M27 and M38.

A CLSI supplement for global application.

M59-Ed3
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Replaces M59-Ed2

Epidemiological Cutoff Values for Antifungal Susceptibility Testing

Gary W. Procop, MD, MS
Philippe J. Dufresne, PhD, RMCCM
Elizabeth Berkow, PhD
Mariana Castanheira, 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 M59—*Epidemiological Cutoff Values for Antifungal Susceptibility Testing* includes epidemiological cutoff values (ECVs) and quality control tables developed following the guidance in CLSI document M57.[1] These ECVs are valid only when they are developed in accordance with CLSI document M57[1] and when minimal inhibitory concentrations or minimal effective concentrations are generated according to the reference broth dilution methods described in CLSI documents M27[2] and M38.[3] Users should replace previously published tables with these new tables. Changes in the tables since the previous edition appear in boldface type.

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Foreword

With the development of standard methodologies for testing the susceptibility of fungal species to several antifungal agents, minimal inhibitory concentration (MIC) and minimal effective concentration (MEC) distributions are available to determine epidemiological cutoff values (ECVs) for *Candida* spp., *Cryptococcus* spp., and *Aspergillus* spp. The ECVs provided in this document were established using the guidance in CLSI document M57.[1] The ECV, which is the MIC or MEC value that defines the upper limit of the wild-type (WT) distribution, is useful for distinguishing between WT isolates without acquired resistance mechanisms and non-WT isolates harboring acquired resistance mechanisms. Unlike breakpoints, ECVs do not classify isolates as treatable (susceptible) or nontreatable (resistant). In lieu of breakpoints, clinicians can use ECVs alone when deciding whether to treat a patient with a certain agent (see CLSI document M57[1]). However, ECVs do not predict therapeutic response. For ECVs to be clinically useful, the MIC or MEC should be determined using the broth microdilution procedure for yeasts (see CLSI document M27[2]) or the broth microdilution procedure for filamentous fungi (see CLSI document M38[3]).

Overview of Changes

This document replaces the previous edition of the approved document, M59-Ed2, published in 2018. Several changes were made in this edition, including:

- **Table 1. Epidemiological Cutoff Values for *In Vitro* Susceptibility Testing of *Candida* spp. With No Breakpoints:**

  NOTE: New *Candida* ECVs were adopted by the Subcommittee on Antifungal Susceptibility Tests during the annual meetings in January 2019 and January 2020. These ECVs are tentative and are open for comment for one year from the publication of M59.

  - Added ECVs for:
    - Amphotericin B
      - *Candida dubliniensis*
      - *Candida guilliermondii*
      - *Candida kefyr*
      - *Candida lusitaniae*
      - *Candida metapsilosis*
      - *Candida orthopsilosis*
    - Anidulafungin
      - *Candida duobushaemulonii*
      - *C. kefyr*
      - *C. metapsilosis*
      - *C. orthopsilosis*
    - Caspofungin
      - *C. duobushaemulonii*
      - *C. lusitaniae*
      - *C. metapsilosis*
      - *C. orthopsilosis*

- Fluconazole
  - *C. duobushaemulonii*
  - *C. kefyr*
  - *C. metapsilosis*
  - *C. orthopsilosis*

- Itraconazole
  - *C. dubliniensis*
  - *C. duobushaemulonii*
  - *C. guilliermondii*
  - *C. kefyr*
  - *C. metapsilosis*
  - *C. orthopsilosis*
  - *Candida parapsilosis*

- Isavuconazole
  - *C. duobushaemulonii*

- Micafungin
  - *C. duobushaemulonii*
  - *C. kefyr*
  - *C. metapsilosis*
  - *C. orthopsilosis*

- Posaconazole
  - *C. dubliniensis*
  - *C. duobushaemulonii*
  - *C. kefyr*
  - *C. metapsilosis*
  - *C. orthopsilosis*

- Voriconazole
  - *C. duobushaemulonii*
  - *C. metapsilosis*
  - *C. orthopsilosis*

– Revised ECV for *C. parapsilosis sensu stricto* for:
  - Amphotericin B

– Added NOTE regarding *Candida* spp. that are *sensu stricto*

– ***Deleted*** footnotes regarding ECVs for *C. parapsilosis* species complex and on fluconazole, posaconazole, and voriconazole ECVs

- **Table 2. Epidemiological Cutoff Values for *In Vitro* Susceptibility Testing of *Cryptococcus* spp. With No Breakpoints:**
  – Revised:
    - Nomenclature for "*C. gattii* (VGII)" to "*C. deuterogattii* (formerly *C. gattii*) (VGII)"

    - Footnote regarding molecular genotypes of *Cryptococcus* spp.

- **Table 3. Epidemiological Cutoff Values for *In Vitro* Susceptibility Testing of *Aspergillus* spp. With No Breakpoints:**
  - Moved ECV for *Aspergillus fumigatus* to Table 5 (new table) for:
    - Voriconazole

- **Table 4. Epidemiological Cutoff Values for *In Vitro* Susceptibility Testing of *Candida* spp. With Breakpoints:**

  NOTE: New *Candida* ECVs (and revised ECVs for *C. parapsilosis sensu stricto*) were adopted by the Subcommittee on Antifungal Susceptibility Tests during the annual meetings in January 2019 and January 2020. These ECVs are tentative and are open for comment for one year from the publication of M59.

  - Added caspofungin ECVs for:
    - *C. guilliermondii*
    - *C. parapsilosis*

  - Revised ECVs for *C. parapsilosis sensu stricto* for:
    - Anidulafungin
    - Fluconazole
    - Micafungin

  - ***Deleted*** ECV for *C. parapsilosis* for:
    - Voriconazole

  - Added NOTE regarding *Candida* spp. that are *sensu stricto*

  - Revised footnote regarding ECVs and breakpoints

  - ***Deleted*** footnotes regarding fluconazole and voriconazole ECVs and on ECVs for *C. parapsilosis* species complex

- **Table 5. Epidemiological Cutoff Values for *In Vitro* Susceptibility Testing of *Aspergillus fumigatus* With Breakpoints:**
  - Added new table

  NOTE: The MIC breakpoints and interpretive categories for voriconazole were adopted by the Subcommittee on Antifungal Susceptibility Tests during the annual meeting in January 2019. These MIC breakpoints and interpretive categories are tentative and are open for comment for one year from the publication of CLSI document M61.[4]

- **Table 6. Summary of Available Epidemiological Cutoff Values and/or Breakpoints by Fungal Species:**
  - Added new table

- **Glossary. Antifungal Agent Abbreviation(s), Route(s) of Administration, and Drug Class:**
  - Added flucytosine

Request for antifungal susceptibility testing data from fungal pathogens needed for the development of ECVs to be included in future editions of M59:

The Working Group on Antifungal Epidemiological Cutoff Values is requesting submission of raw antifungal susceptibility testing data for yeasts and filamentous fungi generated using the protocols provided in CLSI documents M27[2] and M38.[3] This request is only for reference broth microdilution and should not include data generated using commercially available panels. Because the data will be combined with data from other laboratories, even a small amount of data is useful, especially for less frequently identified species. All species should be identified using a molecular assay or matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

A standardized worksheet for data submission is available on the CLSI website at https://clsi.org/meetings/sub-antifungal/ecv-data-submission/. This worksheet can also be requested by contacting CLSI at standard@clsi.org. Completed worksheets can be submitted directly to CLSI at standard@clsi.org.

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

Epidemiological cutoff value, minimal effective concentration, minimal inhibitory concentration, non-wild-type, wild-type

Table 1. Epidemiological Cutoff Values for *In Vitro* Susceptibility Testing of *Candida* spp. With No Breakpoints[1]

Antifungal Agent	Species	ECV, µg/mL[a,b,c]
Amphotericin B	*C. albicans*	2
	C. dubliniensis	**0.5**
	C. glabrata	2
	C. guilliermondii	**2**
	C. kefyr	**2**
	C. krusei	2
	C. lusitaniae[d]	**2**
	C. metapsilosis	**1**
	C. orthopsilosis	**2**
	C. parapsilosis	**1**
	C. tropicalis	2
Anidulafungin	*C. dubliniensis*	0.12
	C. duobushaemulonii	**1**
	C. kefyr	**0.25**
	C. lusitaniae	1
	C. metapsilosis	**0.5**
	C. orthopsilosis	**2**
Caspofungin	***C. duobushaemulonii***	**0.25**
	C. lusitaniae	**1**
	C. metapsilosis	**0.25**
	C. orthopsilosis	**1**
Fluconazole	*C. dubliniensis*	0.5
	C. duobushaemulonii	**32**
	C. guilliermondii	8
	C. kefyr	**1**
	C. lusitaniae	1
	C. metapsilosis	**4**
	C. orthopsilosis	**2**
Isavuconazole	***C. duobushaemulonii***	**0.25**
Itraconazole	***C. dubliniensis***	**0.25**
	C. duobushaemulonii	**1**
	C. glabrata	4
	C. guilliermondii	**2**
	C. kefyr	**0.5**
	C. krusei	1
	C. lusitaniae	1
	C. metapsilosis	**1**
	C. orthopsilosis	**0.5**
	C. parapsilosis	**0.5**
	C. tropicalis	0.5
Micafungin	*C. dubliniensis*	0.12
	C. duobushaemulonii	**0.5**
	C. kefyr	**0.125**
	C. lusitaniae	0.5
	C. metapsilosis	**1**
	C. orthopsilosis	**1**

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Table 1. (Continued)

Antifungal Agent	Species	ECV, µg/mL[a,b,c]
Posaconazole	*C. albicans*	0.06
	C. dubliniensis	**0.125**
	C. duobushaemulonii	**1**
	C. glabrata	1
	C. guilliermondii	0.5
	C. kefyr	**0.5**
	C. krusei	0.5
	C. lusitaniae	0.06
	C. metapsilosis	**0.25**
	C. orthopsilosis	**0.25**
	C. parapsilosis	0.25
	C. tropicalis	0.12
Voriconazole	***C. duobushaemulonii***	**0.5**
	C. glabrata	0.25
	C. metapsilosis	**0.06**
	C. orthopsilosis	**0.125**

Abbreviation: ECV, epidemiological cutoff value.

Footnotes

a. The epidemiological cutoff values (ECVs) in M59 were established using broth microdilution as outlined in CLSI document M27.[1] If another methodology is used for susceptibility testing, that method must be validated against broth microdilution before the ECVs are used, just as other methods must be validated before breakpoints established using broth microdilution are used.

b. ECVs capture ≥97.5% of the statistically modeled population. ECVs may overlook potentially resistant isolates (non-wild-type).

c. If the 24-hour growth control shows insufficient growth, it should be incubated for an additional 24 hours.

d. *C. lusitaniae* is not intrinsically resistant to amphotericin B. However, *C. lusitaniae* may develop resistance to amphotericin B *in vivo* during therapy. When phenotypic resistance was noted in studies, the phenotype was only observed using agar gradient strips and was not detected by broth microdilution methods.[7]

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

NOTE 2: All *Candida* species listed are *sensu stricto* except when stated otherwise.

References for Table 1

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

2 Dalhoff A, Ambrose PG, Mouton JW. A long journey from minimum inhibitory concentration testing to clinically predictive breakpoints: deterministic and probabilistic approaches in deriving breakpoints. *Infection.* 2009;37(4):296-305.

3 Pfaller MA, Espinel-Ingroff A, Bustamante B, et al. Multicenter study of anidulafungin and micafungin MIC distributions and epidemiological cutoff values for eight *Candida* species and the CLSI M27-A3 broth microdilution method. *Antimicrob Agents Chemother.* 2014;58(2):916-922.

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Glossary. Antifungal Agent Abbreviation(s), Route(s) of Administration, and Drug Class

Antifungal Agent	Abbreviation(s)[a]	Route(s) of Administration[b]		Drug Class
		PO	IV	
Amphotericin B	AMB		X	Polyene
Anidulafungin	AND		X	Echinocandin
Caspofungin	CAS, CFG		X	Echinocandin
Fluconazole	FLC, FLU, FLS, FCA, FLZ, FZ	X	X	Azole
Flucytosine	**5-FC**	**X**		**Fluorinated pyrimidine**
Isavuconazole	ISA	X	X	Azole
Itraconazole	ITR	X	X[c]	Azole
Micafungin	MCF		X	Echinocandin
Posaconazole	POS, PCO, POC	X	X	Azole
Voriconazole	VRC, VCO, VOC, VO	X	X	Azole

Abbreviations: IV, intravenous; PO, oral.

Footnotes

a. These abbreviations are assigned to one or more diagnostic products in the United States. If no diagnostic product is available, the abbreviation is that of the manufacturer.

b. As available in the United States.

c. Itraconazole is not available for IV administration in the United States.

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

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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**

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