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M61

Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi

This document provides minimal inhibitory concentration breakpoints and quality control tables for the Clinical and Laboratory Standards Institute antifungal susceptibility testing documents M38 and M51.

A CLSI supplement for global application.

Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi

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 M61—*Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi* includes minimal inhibitory concentration and quality control tables developed following the guidance in CLSI documents M38¹ and M51.² The data in the tables are valid only when the methodologies in CLSI documents M38¹ and M51² are followed. 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

The breakpoints and interpretive categories provided in this document are generated using the reference method for antifungal susceptibility testing of filamentous fungi described in CLSI document M38.¹ These methods may be used for:

- Routine antifungal testing of patient isolates to guide therapy and classify isolates as susceptible or resistant to antifungal agents for which clinical breakpoints have been established
- 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 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.

NOTE: Fungal taxonomy has undergone major changes in recent years. The dual (asexual and sexual stages) nomenclature has been abolished, and fungal species are constantly being reclassified and renamed according to improved molecular characterization.³ Species names listed in CLSI document M38¹ were revised to reflect the most recent taxonomic changes (at the time of publication), based on classification by DNA bar coding. Information on updated fungal species classification is publicly available.⁴

Overview of Changes

This document replaces the previous edition of the approved document, M61-Ed1, published in 2017. Several changes were made in this edition, including:

- **Table 1. Minimal Inhibitory Concentration Breakpoints for Select Antifungal Agents Against *Aspergillus fumigatus*:**
 - Added new table

NOTE: The minimal inhibitory concentration (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 M61.

- **Table 2. Recommended 24-Hour Minimal Inhibitory Concentration or Minimal Effective Concentration Limits for Quality Control and Reference Strains Using Broth Microdilution Antifungal Susceptibility Testing Procedures:**
 - Added new table with information from previous edition's Table 1 (which was separated into three QC tables based on incubation time)

NOTE: The MIC QC ranges for manogepix and rezafungin were adopted by the Subcommittee on Antifungal Susceptibility Tests during the annual meeting in January 2020. These QC ranges are tentative and are open for comment for one year from the publication of M61.

- Added QC ranges for:
 - Manogepix
 - *Candida albicans* ATCC® 90028
 - *Candida parapsilosis* ATCC® 22019
 - *Aspergillus flavus* ATCC® 204304
 - Rezafungin
 - *C. parapsilosis* ATCC® 22019
 - *Candida krusei* ATCC® 6258
- Added 24-hour QC ranges originally published in CLSI document M60⁵ for:
 - Amphotericin B
 - *C. parapsilosis* ATCC® 22019
 - *C. krusei* ATCC® 6258
 - Anidulafungin
 - *C. krusei* ATCC® 6258
 - Fluconazole
 - *C. parapsilosis* ATCC® 22019
 - *C. krusei* ATCC® 6258
 - Flucytosine
 - *C. parapsilosis* ATCC® 22019
 - *C. krusei* ATCC® 6258
 - Isavuconazole
 - *C. parapsilosis* ATCC® 22019
 - *C. krusei* ATCC® 6258
 - Itraconazole
 - *C. parapsilosis* ATCC® 22019
 - *C. krusei* ATCC® 6258
 - Ketoconazole
 - *C. parapsilosis* ATCC® 22019
 - *C. krusei* ATCC® 6258
 - Micafungin
 - *C. krusei* ATCC® 6258
 - Posaconazole
 - *C. parapsilosis* ATCC® 22019
 - *C. krusei* ATCC® 6258
 - Voriconazole
 - *C. parapsilosis* ATCC® 22019
 - *C. krusei* ATCC® 6258
- Revised QC ranges for *C. parapsilosis* ATCC® 22019 for:
 - Anidulafungin
 - Micafungin

- Revised “*Paecilomyces variotii* ATCC[®] MYA-3630” to “*Hamigera insecticola* (previously identified as *Paecilomyces variotii*) ATCC[®] MYA-3630”
- Added footnote regarding caspofungin susceptibility testing
- **Deleted** footnote regarding anidulafungin concentration ranges
- **Table 3. Recommended 48-Hour Minimal Inhibitory Concentration or Minimal Effective Concentration Limits for Quality Control and Reference Strains Using Broth Microdilution Antifungal Susceptibility Testing Procedures:**
 - Added new table with information from previous edition’s Table 1 (which was separated into three QC tables based on incubation time)

NOTE: The MIC QC ranges for orolofim and rezafungin were adopted by the Subcommittee on Antifungal Susceptibility Tests during the annual meeting in January 2020. These QC ranges are tentative and are open for comment for one year from the publication of M61.

- Added QC ranges for:
 - Anidulafungin
 - *C. parapsilosis* ATCC[®] 22019
 - Manogepix
 - *C. albicans* ATCC[®] 90028
 - *C. parapsilosis* ATCC[®] 22019
 - *A. flavus* ATCC[®] 204304
 - *A. fumigatus* ATCC[®] MYA-3626
 - Micafungin
 - *C. parapsilosis* ATCC[®] 22019
 - Rezafungin
 - *C. parapsilosis* ATCC[®] 22019
 - *C. krusei* ATCC[®] 6258
 - Orolofim
 - *A. flavus* ATCC[®] MYA-3631
 - *A. fumigatus* ATCC[®] MYA-3626
- Revised QC ranges for *C. parapsilosis* ATCC[®] 22019 for:
 - Itraconazole
 - Posaconazole
- Revised:
 - *Fusarium* spp. (*Fusarium verticillioides* [moniliforme] ATCC[®] MYA-3629 and *Fusarium solani* ATCC[®] MYA-3636) reference ranges from MIC to minimal effective concentration and adjusted the within-range percentage
 - “*Paecilomyces variotii* ATCC[®] MYA-3630” to “*Hamigera insecticola* (previously identified as *Paecilomyces variotii*) ATCC[®] MYA-3630”

- **Table 4. Recommended 48- to 96-Hour Minimal Inhibitory Concentration or Minimal Effective Concentration Limits for Quality Control and Reference Strains Using Broth Microdilution Antifungal Susceptibility Testing Procedures:**
 - Added new table with information from previous edition’s Table 1 (which was separated into three QC tables based on incubation time)
 - *Deleted* footnote regarding anidulafungin concentration ranges
- **Table 5. Recommended Zone Diameter Limits for Quality Control and Reference Strains Using Disk Diffusion Antifungal Susceptibility Testing Procedures:**
 - Corrected reference range for *Paecilomyces variotii* ATCC® MYA-3630 for:
 - Caspofungin
 - Revised “*Paecilomyces variotii* ATCC® MYA-3630” to “*Hamigera insecticola* (previously identified as *Paecilomyces variotii*) ATCC® MYA-3630”
 - *Deleted* footnote regarding tentative QC zone diameter ranges
- **Table 6. Solvents and Diluents for Preparation of Stock Solutions of Antifungal Agents:**
 - Added:
 - Manogepix
 - Orolofim
 - Rezafungin
 - Added footnotes regarding dimethyl sulfoxide and manufacturer recommendations

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, disk diffusion, echinocandin, filamentous fungi, interpretive category, microbroth dilution, minimal inhibitory concentration, quality control, susceptibility testing

Table 1. Minimal Inhibitory Concentration Breakpoints for Select Antifungal Agents Against *Aspergillus fumigatus*

Antifungal Agent	Species	MIC Breakpoints and Interpretive Categories, $\mu\text{g/mL}$		
		S	I	R
Voriconazole ^a	<i>A. fumigatus</i>	≤ 0.5	1	≥ 2

Abbreviations: I, intermediate; MIC, minimal inhibitory concentration; R, resistant; S, susceptible.

Footnote

- a. Interpretive breakpoints were derived from a collection of sequence-confirmed isolates of *A. fumigatus sensu stricto* and are not applicable to other members of the *A. fumigatus* species complex.

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

Table 2. Recommended 24-Hour Minimal Inhibitory Concentration or Minimal Effective Concentration Limits for Quality Control and Reference Strains Using Broth Microdilution Antifungal Susceptibility Testing Procedures

Organism	Purpose	Antifungal Agent	MIC/MEC Range, $\mu\text{g/mL}$	MIC/MEC Mode, $\mu\text{g/mL}$	MICs/MECs Within Range, %
<i>Candida albicans</i> ATCC ^{®a} 90028	QC	Manogepix	0.004–0.015	0.008	100
<i>Candida krusei</i> ATCC [®] 6258 ^{1,2}	QC	Amphotericin B	0.5–2	1	100
		Anidulafungin	0.03–0.12	0.06	97.9
		Caspofungin ^b	0.12–1	0.5	98.8
		Fluconazole	8–64	16	100
		Flucytosine	4–16	8	97.5
		Isavuconazole	0.06–0.5	0.25	95.2
		Itraconazole	0.12–1	0.5	95.8
		Ketoconazole	0.12–1	0.5	95.4
		Micafungin	0.12–0.5	0.25	99.6
		Posaconazole	0.06–0.5	0.25	100
		Rezafungin	0.015–0.12	0.03	100
		Voriconazole	0.06–0.5	0.25	98.3
<i>Candida parapsilosis</i> ATCC [®] 22019 ^{1,2}	QC	Amphotericin B	0.25–2	0.5	97.1
		Anidulafungin	0.25–2	1	95
		Caspofungin	0.25–1	0.5	96.7
		Fluconazole	0.5–4	2	98.2
		Flucytosine	0.06–0.25	0.12	99.2
		Isavuconazole	0.015–0.06	0.06	90.5
		Itraconazole	0.06–0.5	0.25	95.8
		Ketoconazole	0.03–0.25	0.06/0.12	97.5
		Manogepix	0.008–0.03	0.015	100
		Micafungin	0.5–2	1	100
		Posaconazole	0.03–0.25	0.12	96.7
		Rezafungin	0.25–2	0.5	100
		Voriconazole	0.016–0.12	0.06	100
<i>Aspergillus flavus</i> ATCC [®] 204304	QC	Manogepix	0.004–0.03	0.008	99.2
<i>Aspergillus fumigatus</i> ATCC [®] MYA-3626 ³	Reference (MEC)	Anidulafungin	≤ 0.015	N/A	100
<i>Aspergillus terreus</i> ATCC [®] MYA-3633 ³	Reference (MEC)	Anidulafungin	≤ 0.015	N/A	99.6
<i>Hamigera insecticola</i> (previously identified as <i>Paecilomyces variotii</i>) ATCC [®] MYA-3630 ³	Reference (MEC)	Anidulafungin	≤ 0.015	N/A	100

Abbreviations: ATCC[®], American Type Culture Collection; MEC, minimal effective concentration; MIC, minimal inhibitory concentration; N/A, not applicable; QC, quality control.

Footnotes

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

Table 3. (Continued)

Organism	Purpose	Antifungal Agent	MIC/MEC Range, µg/mL	MIC/MEC Mode, µg/mL	MICs/MECs Within Range, %
<i>Aspergillus fumigatus</i> ATCC® MYA-3626 ⁵	Reference	Amphotericin B	0.5–4	2	98.7
		Itraconazole	0.25–2	1	95.7
		Voriconazole	0.25–1	0.5	100
	QC	Manogepix	0.008–0.06	0.03	98.6
		Orolofim	0.008–0.06	0.03	98.2
<i>A. fumigatus</i> ATCC® MYA-3627 ⁵	Reference	Amphotericin B	0.5–4	2	99.2
		Itraconazole	≥16	>16	95
		Voriconazole	0.25–1	0.5	99.2
<i>Aspergillus terreus</i> ATCC® MYA-3633 ⁵	Reference	Amphotericin B	2–8	4	98.3
		Voriconazole	0.25–1	0.5	99.2
<i>Fusarium solani</i> ATCC® MYA-3636 ⁶	Reference (MEC) ^d	Anidulafungin	>8	N/A	87
<i>Fusarium verticillioides</i> (moniliforme) ATCC® MYA-3629 ^{5,6}	Reference	Amphotericin B	2–8	4	99.6
		Itraconazole	>16	>16	97.9
		Posaconazole	0.5–2	1	98.1
		Voriconazole	1–4	2	100
	Reference (MEC) ^d	Anidulafungin	>8	N/A	87.5
<i>Hamigera insecticola</i> (previously identified as <i>Paecilomyces variotii</i>) ATCC® MYA-3630 ⁵	QC	Amphotericin B	1–4	2	100
		Isavuconazole	0.06–0.5	0.12	96.7
		Itraconazole	0.06–0.5	0.12	100
		Posaconazole	0.03–0.25	0.06	99.5
		Voriconazole	0.015–0.12	0.06	100

Abbreviations: ATCC®, American Type Culture Collection; MEC, minimal effective concentration; MIC, minimal inhibitory concentration; N/A, not applicable; ND, not determined; QC, quality control.

Footnotes

- ATCC® is a registered trademark of the American Type Culture Collection.
- The percentage of manogepix minimal inhibitory concentrations (MICs) within range for *C. parapsilosis* ATCC® 22019 and *A. flavus* ATCC® 204304 was less than the threshold value of 95% necessary for quality control isolates. These values may be assessed and adjusted in the future based on Tier 3 data.
- The MIC ranges for *A. flavus* ATCC® 204304 are based on data from a collaborative study^{3,4} that were not obtained according to the process described in CLSI document M23.⁷ However, *A. flavus* is the only mold for which reproducible reference limits for ravuconazole have been established, so it is included in this table.
- Although the anidulafungin concentration range in the study was 0.015 to 32 µg/mL, off-scale MICs >32 µg/mL from that study are reported in this table as >8 µg/mL for consistency with the recommended routine testing range for this compound.⁶

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

References for Table 3

- Barry AL, Pfaller MA, Brown SD, et al. Quality control limits for broth microdilution susceptibility tests of ten antifungal agents. *J Clin Microbiol.* 2000;38(9):3457-3459.
- Krisher K, Brown SD, Traczewski MM. Quality control parameters for broth microdilution tests of anidulafungin. *J Clin Microbiol.* 2004;42(1):490.

Sample



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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