CLINICAL AND LABORATORY STANDARDS INSTITUTE®

1st Edition

M61

Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi

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

A CLSI supplement for global application.

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Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi

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Abstract

Clinical and Laboratory Standards Institute document M61—*Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi* provides information to use with the testing procedures in CLSI documents M38[1] and M51.[2]

The tabular information in this document includes the most current information for antifungal agent selection, results interpretation, and quality control and is valid only when the methodology is followed as described in the current editions of CLSI documents M38[1] and M51.[2] Any previously published tables should be replaced with these new tables. Changes since the last edition appear in boldface type.

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Contents

Foreword

Users of CLSI documents M38[1] and M51[2] and this document should recognize that the standard methods described in CLSI documents are reference methods. These methods may be used for:

- Routine antifungal testing of patient isolates to guide therapy
- Evaluating commercial devices that will be used in medical laboratories
- Testing new agents or systems by drug or device manufacturers

Results generated by reference methods, such as those provided in CLSI documents, may be used by regulatory authorities to evaluate commercial susceptibility testing device performance as part of the 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 with the dual (asexual and sexual stages) nomenclature having been abolished and the constant reclassification and renaming of fungal species that result from improved molecular characterization.[3] Species names listed in CLSI document M38[1] were revised to reflect the most recent taxonomic changes based on classification according to DNA bar coding at the time of publication. For more information regarding updated fungal species reclassification, refer to publicly available information.[4]

Overview of Changes

This document replaces the previous edition of the approved supplement M51-S1, published in 2010, and incorporates information from CLSI document M38-A2. Several changes were made in this edition, including:

- **General:**
  - Combined supplemental information from M38-A2 (broth dilution susceptibility testing of filamentous fungi) and M51-S1 (disk diffusion testing of filamentous fungi) into one informational supplement, which has been recoded as M61
  - Deleted the glossary with abbreviations, routes of administration, and drug classes for antifungal agents
- **Tables:**
  - Moved Table 1 from M38-A2 (Solvents and Diluents for Preparation of Stock Solutions of Antifungal Agents) to M61, where it is now Table 3, and added information for isavuconazole
  - Updated QC ranges for itraconazole and posaconazole for *Candida parapsilosis* and added QC ranges for isavuconazole in Table 1
  - Moved Table 4 from M38-A2 (Recommended MIC or MEC Limits for QC and Reference Strains for Broth Dilution Procedures) to M61, where it is now Table 1, and revised the title (Recommended Minimal Inhibitory Concentration or Minimal Effective Concentrations and Modes for Quality Control and Reference Strains for Broth Dilution Antifungal Susceptibility Testing Procedures)
  - Moved Table 1 from M51-S1 (Tentative Zone Diameter Epidemiological Cutoff Values [ECV] and Corresponding Minimal Inhibitory Concentration [MIC] or Minimal Effective Concentration [MEC] for Filamentous Fungi) to CLSI document M59,[5] the supplement to CLSI document M57[6]

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- Moved Table 2 from M51-S1 (Recommended Reference Control Zone Diameter [mm] Ranges) to M61 (where it is also Table 2 and now titled Recommended Zone Diameter Limits and Modes for Quality Control and Reference Strains for Disk Diffusion Antifungal Susceptibility Testing Procedures) and revised with additional QC data

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, disk diffusion, echinocandin, filamentous fungi, microbroth dilution, quality control, susceptibility testing

Abbreviations and Acronyms

ATCC®[a]	American Type Culture Collection
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
MEC	minimal effective concentration
MIC	minimal inhibitory concentration
QC	quality control

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

Table 1. Recommended Minimal Inhibitory Concentration or Minimal Effective Concentrations and Modes for Quality Control and Reference Strains for Broth Dilution Antifungal Susceptibility Testing Procedures

Organism	Purpose	Antifungal Agent	MIC/MEC Range, µg/mL	MIC/ MEC Mode, µg/mL	MICs/ MECs Within Range, %	Incubation Time, hours*
Paecilomyces variotii ATCC®† MYA-3630[1,2]	QC	Amphotericin B	1–4	2	100	48
		Isavuconazole	**0.06–0.5**	**0.12**	**96.7**	**48**
		Itraconazole	0.06–0.5	0.12	100	48
		Posaconazole	0.03–0.25	0.06	99.5	48
		Voriconazole	0.015–0.12	0.06	100	48
	Reference (MEC)‡	Anidulafungin	≤0.015	N/A	100	24
Candida parapsilosis ATCC® 22019[3,4]	QC	Amphotericin B	0.5–4	2	91.7	48
		Anidulafungin	0.5–2	1	95	24
		Caspofungin	0.25–1	0.5	96.7	24
		Caspofungin	0.5–4	1	92.9	48
		Fluconazole	1–4	2	98.1	48
		Flucytosine	0.12–0.5	0.25	97.9	48
		Isavuconazole	**0.03–0.12**	**0.03**	**98.3**	**48**
		Itraconazole	**0.06**–0.5	0.25	97.5	48
		Ketoconazole	0.06–0.5	0.12	98.3	48
		Micafungin	0.5–4	1	100	24
		Posaconazole	**0.03**–0.25	0.12	98.8	48
		Ravuconazole	0.03–0.25	0.06	98.3	48
		Voriconazole	0.03–0.25	0.06	100	48
Candida krusei ATCC® 6258[3,4]	QC	Amphotericin B	1–4	2	100	48
		Anidulafungin	0.03–0.12	0.06	97.5	48
		Caspofungin	0.12–1	0.5	98.8	24
		Caspofungin	0.25–1	0.5	97.5	48
		Fluconazole	16–128	32	100	48
		Flucytosine	8–32	16	99.6	48
		Isavuconazole	**0.12–0.5**	**0.12**	**94.2**	**48**
		Itraconazole	0.25–1	0.5	100	48
		Ketoconazole	0.25–1	0.5	99.6	48
		Micafungin	0.12–0.5	0.25	99	48
		Posaconazole	0.12–1	0.5	99.6	48
		Ravuconazole	0.25–1	0.5	100	48
		Voriconazole	0.12–1	0.5	100	48
Aspergillus flavus ATCC® 204304[5,6,§]	Reference	Amphotericin B	0.5–4	ND	100	48
		Itraconazole	0.25–0.5	ND	100	48
		Posaconazole	0.06–0.5	ND	100	48
		Ravuconazole	0.5–4	ND	100	48
		Voriconazole	0.5–4	ND	100	48
	QC	**Isavuconazole**	**0.5–4**	**1**	**95.8**	**48**
A. flavus ATCC® MYA-3631[1]	Reference	Amphotericin B	1–8	2	98.8	48
		Posaconazole	0.12–1	0.5	97.1	48
		Voriconazole	0.5–2	1	98.3	48
Aspergillus fumigatus ATCC® MYA-3626[1,2]	Reference	Amphotericin B	0.5–4	2	98.7	48
		Itraconazole	0.25–2	1	95.7	48
		Voriconazole	0.25–1	0.5	100	48
	Reference (MEC)‡	Anidulafungin	≤0.015	N/A	100	24

Table 1. (Continued)

Organism	Purpose	Antifungal Agent	MIC/MEC Range, µg/mL	MIC/ MEC Mode, µg/mL	MICs/ MECs Within Range, %	Incubation Time, hours*
A. fumigatus ATCC® MYA-3627[1]	Reference	Amphotericin B	0.5–4	2	99.2	48
		Itraconazole	≥16	≥16	95	48
		Voriconazole	0.25–1	0.5	99.2	48
Aspergillus terreus ATCC® MYA-3633[1,2]	Reference	Amphotericin B	2–8	4	98.3	48
		Voriconazole	0.25–1	0.5	99.2	48
	Reference (MEC)‡	Anidulafungin	≤0.015	N/A	99.6	24
Fusarium verticillioides (moniliforme) ATCC® MYA-3629[1,2]	Reference	Amphotericin B	2–8	4	99.6	48
		Itraconazole	>16	>16	97.9	48
		Posaconazole	0.5–2	1	98.1	48
		Voriconazole	1–4	2	100	48
F. verticillioides (moniliforme) ATCC®† MYA-3629[1,2]	Reference (MIC)‡	Anidulafungin	>8	N/A	97.5	48
Fusarium solani ATCC® MYA-3636[2]	Reference (MIC)‡	Anidulafungin	>8	N/A	96.7	48
Scedosporium apiospermum ATCC® MYA-3635[5,6]	Reference	Amphotericin B	4–16	8	98.8	72
		Posaconazole	1–4	2	98.3	72
		Voriconazole	0.5–2	1	100	72
S. apiospermum ATCC® MYA-3634[2]	Reference (MEC)‡	Anidulafungin	1–4	2	96.7	48–72
Trichophyton mentagrophytes¶ MRL 1957 ATCC® MYA-4439[7]	Reference	Ciclopirox	0.5–2	1	97.5	96
		Griseofulvin	0.12–0.5	0.25	96.3	96
		Itraconazole	0.03–0.25	0.06	96.2	96
		Posaconazole	0.03–0.25	0.06	95.2	96
		Terbinafine	0.002–0.008	0.004	97.9	96
		Voriconazole	0.03–0.25	0.06	95.2	96
Trichophyton rubrum¶ MRL 666 ATCC® MYA-4438[7]	Reference	Ciclopirox	0.5–2	1	97.5	96
		Fluconazole	0.5–4	1	95.2	96
		Voriconazole	0.008–0.06	0.015	96.1	96

* MIC ranges correspond to the indicated incubation time. In some cases, MIC ranges are also available by the macrodilution method (48 hours only) and after 24 hours by the microdilution method.[8-10] One of the QC isolates should be used per standard QC testing procedures (see CLSI document M38[11]).

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

‡ 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 Table 1 as >8 µg/mL to be consistent with the recommended routine testing range for this compound.[2]

§ The MIC ranges for *A. flavus* ATCC® 204304 are based on data from a collaborative study[5,6] that were not obtained according to the CLSI document M23[12] process. However, *A. flavus* is the only mould for which reproducible reference limits for ravuconazole have been established, so it is included in Table 1.

¶ Ninety-six hours or until good growth (confluent hyphal growth covering the bottom of the well) is obtained in the growth control well.[13]

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

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