CLINICAL AND LABORATORY STANDARDS INSTITUTE®

3rd Edition

M38M51S

Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi

This document includes 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.

M38M51S-Ed3
August 2022
Replaces M61-Ed2

Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi

Gary W. Procop, MD, MS
Philippe J. Dufresne, PhD, RMCCM
Elizabeth Berkow, PhD
Sharon K. Cullen, BS, RAC
Jeff Fuller, PhD, FCCM, D(ABMM)
Kimberly E. Hanson, MD, MHS
Nicole M. Holliday, BA
Sixto M. Leal, MD, PhD
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 M38M51S—*Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi* includes minimal inhibitory concentration and quality control tables developed following the guidance in CLSI documents M38[1] and M51.[2] The data in the tables are valid only when the methodologies in CLSI documents M38[1] and M51[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.

Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi*. 3rd ed. CLSI supplement M38M51S (ISBN 978-1-68440-160-4 [Print]; ISBN 978-1-68440-161-1 [Electronic]). Clinical and Laboratory Standards Institute, USA, 2022.

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

Suggested Citation

CLSI. *Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi*. 3rd ed. CLSI supplement M38M51S. Clinical and Laboratory Standards Institute; 2022.

Previous Editions:
M51-S1: May 2010
M61: November 2017, June 2020

M38-Ed3-M51-Ed1-S-Ed3
ISBN 978-1-68440-160-4 (Print)
ISBN 978-1-68440-161-1 (Electronic)
ISSN 1558-6502 (Print)
ISSN 2162-2914 (Electronic)

Volume 42, Number 19

Contents

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 documents M38[1] **and M51.**[2] 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 documents M38[1] **and M51**[2] 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.[4-7]

NOTE: When serial twofold dilution MICs are being prepared and tested, the actual dilution scheme is, eg, 128, 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 M38M51S: 128, 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.

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

Antifungal Agent	MIC Breakpoints and Interpretive Categories, µg/mL		
	S	I	R
Voriconazole[a]	≤ 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.

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

General Comment

(1) For manogepix, olorofim, oteseconazole, and rezafungin, reading criteria are not included in CLSI document M38[1]; however, these agents will be included in the next edition. For these antifungal agents, the MIC/MEC reading criteria below should be applied for yeasts and molds. For yeasts, the MICs for manogepix, oteseconazole, and rezafungin are read at the lowest concentration at which a score of 2 is observed (prominent decrease in turbidity or > 50% inhibition of growth compared with the growth control; see CLSI document M27[2]). For molds, manogepix and rezafungin should be read according to the MEC (see CLSI document M38[1]). For olorofim, the MIC is the lowest concentration at which complete (100%) inhibition of growth compared with the growth control is observed.

Organism	Purpose	Antifungal Agent	MIC/MEC Range, µg/mL	MIC/MEC Mode, µg/mL	MICs/MECs Within Range, %
Candida albicans ATCC®[a] 90028	QC	Manogepix	0.004-0.016	0.008	100
		Oteseconazole[b]	**0.002-0.016**	**0.004**	**95.3**
Candida krusei ATCC® 6258[3,4]	QC	Amphotericin B	0.5-2	1	100
		Anidulafungin	0.03-0.12	0.06	97.9
		Caspofungin[c]	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	**0.06-0.25**	**0.12-0.25**	99.6
		Posaconazole	0.06-0.5	0.25	100
		Rezafungin	0.016-0.12	0.03	100
		Voriconazole	0.06-0.5	**0.12**	98.3

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

Organism	Purpose	Antifungal Agent	MIC/MEC Range, µg/mL	MIC/ MEC Mode, µg/mL	MICs/ MECs Within Range, %	Incubation Time, hours
Scedosporium apiospermum ATCC®[a] MYA-3635[1,2]	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[3]	Reference (MEC)	Anidulafungin	1-4	2	96.7	48-72
Trichophyton mentagrophytes[b] MRL 1957 ATCC® MYA-4439[4]	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[b] MRL 666 ATCC® MYA-4438[4]	Reference	Ciclopirox	0.5-2	1	97.5	96
		Fluconazole	0.5-4	1	95.2	96
		Voriconazole	0.008-0.06	0.016	96.1	96

Abbreviations: ATCC®, American Type Culture Collection; MEC, minimal effective concentration; MIC, minimal inhibitory concentration.

Footnotes

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

b. This strain should be incubated for 96 hours or until confluent hyphal growth covers the bottom of the growth control well.[4]

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

PRINT ISBN 978-1-68440-160-4

ELECTRONIC ISBN 978-1-68440-161-1

M38-Ed3-M51-Ed1-S-Ed3