



M38

Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi

This standard includes antifungal agent selection, preparation of antifungal stock solutions and dilutions for testing, test procedure implementation and interpretation, and quality control requirements for susceptibility testing of filamentous fungi (moulds) that cause invasive and cutaneous fungal infections.

A standard for global application developed through the Clinical and Laboratory Standards Institute consensus process.

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Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi

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Abstract

Clinical and Laboratory Standards Institute standard M38—*Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi* describes a method for testing the susceptibility to antifungal agents of filamentous fungi (nondermatophyte and dermatophyte moulds) that cause invasive and/or cutaneous fungal infections. Antifungal agent selection, preparation of antifungal stock solutions and dilutions for testing, test procedure implementation and interpretation, and the purpose and implementation of QC procedures are discussed. A careful examination of manufacturer and user responsibilities in QC is also presented. In addition, a brief discussion regarding newly defined epidemiological cutoff values for certain *Aspergillus* spp. and species complexes are included.

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SAMPLE

Foreword

With the increased incidence of systemic fungal infections and the growing number of available antifungal agents, laboratory guidance for selecting antifungal therapy has gained greater attention. The Subcommittee on Antifungal Susceptibility Tests concluded that a reproducible reference procedure for the antifungal susceptibility testing of filamentous fungi (moulds) would be useful. Accordingly, several studies were conducted to refine the methodology for performing nondermatophyte mould susceptibility testing.¹⁻⁵ The resulting consensus method was published in 2002 as M38, and a revision published in 2008.

In the previous edition of this standard, supplemental material (QC data for mould isolates as well as echinocandin testing guidelines) was incorporated and guidelines for testing dermatophyte moulds were provided.⁵⁻¹⁰ Since then, in the absence of breakpoints for mould testing, epidemiological cutoff values (ECVs) for distinguishing wild-type and non-wild-type isolates (those with intrinsic or acquired known resistance mechanisms or gene mutations) have been defined for some species and species complexes of *Aspergillus* (see CLSI documents M57¹¹ and M59¹²).¹³⁻¹⁷ Although a discussion regarding breakpoints was introduced in the previous edition of M38, breakpoints have not been established by CLSI for mould testing. ECV data and recommendations for their development are found in CLSI documents M57¹¹ and M59.¹² QC data for testing mould isolates, as well as other testing guidelines, have been omitted from this edition of M38 and incorporated into the newly created CLSI document M61,¹⁸ which combines supplemental material for this standard and CLSI document M51.¹⁹

Overview of Changes

This standard replaces the previous edition of the approved standard, M38-A2, published in 2008. Several changes were made in this edition, including:

- **General:**
 - Revised document format and organization to reflect the CLSI quality system essential and path of workflow document templates and the updated CLSI style
 - Updated references to the previous informational supplement (M51-S1) to reflect CLSI document M61,¹⁸ the new supplement for broth dilution and disk diffusion mould susceptibility testing
 - Added references to epidemiological cutoff values and CLSI documents M57¹¹ and M59¹²
- **Subchapter 1.4.2, Definitions:**
 - Revised the breakpoint and interpretive category definitions for consistency with other CLSI antimicrobial susceptibility testing documents.
 - Added definitions for “wild-type” and “non-wild-type”
- **Chapter 2, Preparing for Antifungal Susceptibility Testing:**
 - Added new indications for testing of filamentous fungi, with a discussion of resistance in *Aspergillus fumigatus* originating from the natural environment
- **Chapter 3, Antifungal Broth Dilution Susceptibility Testing Process for Filamentous Fungi:**
 - Added an antifungal susceptibility testing process flow chart
 - Expanded the list of relevant drug concentrations to be tested for echinocandins
 - Replaced procedural text with step-action tables

M38, 3rd ed.

- Established guide for reading and interpreting results for filamentous fungi, including dermatophytes
- Modified text on reading results in Subchapter 3.4 to include new information on echinocandins and isavuconazole antifungal agents and minimal inhibitory concentration (MIC) and minimal effective concentration (MEC) comparison
- **Subchapter 4.6, Quality Control Frequency:**
 - Added a note for the preparation of *Candida* spp. QC strains (Subchapter 4.6.1)
- **Appendixes (Original Tables):**
 - Updated and moved the solvent list table from M38 to the new supplement, CLSI document M61¹⁸
 - Moved the table providing the recommended MIC or MEC limits for QC and reference strains for broth dilution procedures from M38 to the combined supplement, CLSI document M61¹⁸
 - Corrected Appendix C (Composition of Roswell Park Memorial Institute 1640 Culture Medium) to provide a single riboflavin concentration (0.0002 g/L), as found in CLSI document M27²⁰
 - Harmonized dilution schemes for dermatophyte and nondermatophyte isolates with those in CLSI document M27²⁰ and revised to encompass the full dilution ranges recommended
 - Deleted the procedure for preparing a 0.5 McFarland (barium sulfate) standard and added a note referring to CLSI document M27²⁰ for *Candida* spp. QC strains to Subchapter 4.6.1

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

Key Words

Antifungal agent, broth microdilution, dermatophytes, epidemiological cutoff value, filamentous fungi, mould, non-wild-type, susceptibility testing, wild-type

Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi

Chapter 1: Introduction

This chapter includes:

- Standard's scope and applicable exclusions
- Background information pertinent to the standard's content
- Standard precautions information
- "Note on Terminology" that highlights particular use and/or variation in use of terms and/or definitions
- Terms and definitions used in the standard
- Abbreviations and acronyms used in the standard

1.1 Scope

This standard describes the reference broth microdilution testing method for antifungal susceptibility testing of filamentous fungi (moulds) that cause invasive and/or cutaneous fungal infections.¹⁻¹⁰ This standard also covers testing conditions, including inoculum preparation and inoculum size, incubation time and temperature, media formulation, and end-point determination criteria.¹⁻⁹ QC reference ranges and limits and specific epidemiological cutoff values (ECVs) are summarized in the current editions of CLSI documents M61¹⁸ and M59,¹² respectively.^{5,8-10,13-17}

The intended audience includes medical laboratory personnel, clinicians, and microbiologists who routinely perform antifungal susceptibility testing and use antifungal susceptibility testing results to select suitable antifungal therapy, as well as those involved in emerging resistance surveillance. The reference method is also useful for establishing ECVs and developing and validating alternate commercial methods for determining antifungal susceptibility of filamentous fungi. Therefore, the standard is also of interest for both diagnostic and pharmaceutical companies and their regulatory counterparts.

This method has not been evaluated in studies of the yeast or mould forms of dimorphic fungi, such as *Blastomyces dermatitidis*, *Coccidioides immitis/posadasii*, *Histoplasma capsulatum*, or *Talaromyces marneffei* (*Penicillium marneffei*), and has been evaluated only for the mycelial form of *Sporothrix schenckii* species complex.¹ This method also has not been used in studies of dermatophytes with the echinocandins or nondermatophyte moulds with ciclopirox, griseofulvin, or terbinafine.

Antifungal susceptibility testing of other filamentous fungi that cause infections may also be tested by this method but have not been standardized and evaluated in collaborative studies. The appropriate testing parameters such as inoculum and incubation time for those fungi are unknown.

Commercially available susceptibility test systems are out of scope for this standard. It is recommended that users of these systems refer to the manufacturer's instructions as outlined in the package insert.

1.2 Background

The method described in this standard is intended for testing common filamentous fungi, including the dermatophytes (*Trichophyton* spp., *Microsporum* spp., and *Epidermophyton* spp.),⁹ more prevalent *Aspergillus* spp. and species complexes, *Fusarium* spp. and species complexes, *Rhizopus* spp. and other Mucorales (Zygomycetes),²¹ *Lomentospora prolificans* (*Scedosporium prolificans*),²² the mycelial form of species included in the *S. schenckii* complex, and dematiaceous (phaeoid [black]) moulds.²³

This standard provides a reference method developed through a consensus process to facilitate agreement among laboratories in measuring mould susceptibility to antifungal agents. An important use of a reference method is to provide a standard basis from which other methods can be developed, which also results in interlaboratory agreement within specified ranges. Such methods might have particular advantages, such as ease of performance, economy, or production of more rapid results; therefore, their development could be highly desirable. To the extent that any method produces results concordant with this reference method, it would be considered to conform to this standard.

1.3 Standard Precautions

Because it is often impossible to know what isolates or specimens might be infectious, all patient and laboratory specimens are treated as infectious and handled according to “standard precautions.” Standard precautions are guidelines that combine the major features of “universal precautions and body substance isolation” practices. Standard precautions cover the transmission of all known infectious agents and thus are more comprehensive than universal precautions, which are intended to apply only to transmission of bloodborne pathogens. Published guidelines are available that discuss the daily operations of diagnostic medicine in humans and animals while encouraging a culture of safety in the laboratory.²⁴ For specific precautions for preventing the laboratory transmission of all known infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all known infectious diseases, refer to CLSI document M29.²⁵

1.4 Terminology

1.4.1 A Note on Terminology

CLSI, as a global leader in standardization, is firmly committed to achieving global harmonization whenever possible. Harmonization is a process of recognizing, understanding, and explaining differences while taking steps to achieve worldwide uniformity. CLSI recognizes that medical conventions in the global metrological community have evolved differently in different countries and regions and that legally required use of terms, regional usage, and different consensus timelines are all important considerations in the harmonization process. CLSI recognizes its important role in these efforts, and its consensus process focuses on harmonization of terms to facilitate the global application of standards and guidelines.

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.²⁶ Species names listed in M38 are 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.²⁷

NOTE: At the time of this standard’s publication, breakpoints are not yet available for any antifungal agent against mould species; however, ECVs based solely on *in vitro* data have been established (see CLSI document M59¹²). Currently, breakpoints apply only to some *Candida* spp. and antifungal agent combinations. Definitions for breakpoints and interpretive categories are provided in Subchapter 1.4.2 in the event that breakpoints are published for filamentous fungi in the future.

The Quality Management System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management system (QMS) approach in the development of standards and guidelines that facilitates project management, defines a document structure using a template, and provides a process to identify needed documents. The QMS approach applies a core set of “quality system essentials” (QSEs), basic to any organization, to all operations in any health care service’s path of workflow (ie, operational aspects that define how a particular product or service is provided). The QSEs provide the framework for delivery of any type of product or service, serving as a manager’s guide. The QSEs are:

Organization	Personnel	Process Management	Nonconforming Event Management
Customer Focus	Purchasing and Inventory	Documents and Records	Assessments
Facilities and Safety	Equipment	Information Management	Continual Improvement

M38 covers the QSE indicated by an “X.” For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section.

Organization	Customer Focus	Facilities and Safety	Personnel	Purchasing and Inventory	Equipment	Process Management	Documents and Records	Information Management	Nonconforming Event Management	Assessments	Continual Improvement
		M29 M54				X M07 M23 M27 M51 M54 M57 M59 M61					

Path of Workflow

A path of workflow is the description of the necessary processes to deliver the particular product or service that the organization or entity provides. A laboratory path of workflow consists of the sequential processes: preexamination, examination, and postexamination and their respective sequential subprocesses. All laboratories follow these processes to deliver their services, namely quality laboratory information.

M38 covers the medical laboratory path of workflow processes indicated by an “X.” For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section.

Preexamination				Examination			Postexamination	
Examination ordering	Sample collection	Sample transport	Sample receipt and processing	Examination	Results review and follow-up	Interpretation	Results reporting and archiving	Sample management
	M54	M54	M54	X M07 M27 M51 M54	X M07 M27 M51 M59	X M07 M27 M51 M54 M59	X M07 M27 M51 M59	X M27 M51

Related CLSI Reference Materials*

- M07** **Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. 10th ed., 2015.** This standard covers reference methods for determining minimal inhibitory concentrations of aerobic bacteria by broth macrodilution, broth microdilution, and agar dilution.
- M23** **Development of *In Vitro* Susceptibility Testing Criteria and Quality Control Parameters. 4th ed., 2016.** This guideline discusses the necessary and recommended data for selecting appropriate breakpoints and quality control ranges for antimicrobial agents.
- M27** **Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts. 4th ed., 2017.** This standard covers antifungal agent selection and preparation, test procedure implementation and interpretation, and quality control requirements for susceptibility testing of yeasts that cause invasive fungal infections.
- M29** **Protection of Laboratory Workers From Occupationally Acquired Infections. 4th ed., 2014.** Based on US regulations, this document provides guidance on the risk of transmission of infectious agents by aerosols, droplets, blood, and body substances in a laboratory setting; specific precautions for preventing the laboratory transmission of microbial infection from laboratory instruments and materials; and recommendations for the management of exposure to infectious agents.
- M51** **Method for Antifungal Disk Diffusion Susceptibility Testing of Nondermatophyte Filamentous Fungi. 1st ed., 2010.** This document describes the guidelines for antifungal susceptibility testing by the disk diffusion method of nondermatophyte filamentous fungi (moulds) that cause invasive disease.
- M54** **Principles and Procedures for Detection of Fungi in Clinical Specimens—Direct Examination and Culture. 1st ed., 2012.** This guideline provides protocols, quality control parameters, and interpretive criteria for performing fungal cultures and for the detection and identification of fungi in direct examinations.
- M57** **Principles and Procedures for the Development of Epidemiological Cutoff Values for Antifungal Susceptibility Testing. 1st ed., 2016.** This guideline includes the criteria for developing and using epidemiological cutoff values for guiding clinical decisions when testing fungal species and antifungal agent combinations for which there are no breakpoints.
- M59** **Epidemiological Cutoff Values for Antifungal Susceptibility Testing. 1st ed., 2016.** This document provides the epidemiological cutoff value and quality control tables developed according to criteria provided in the Clinical and Laboratory Standards Institute guideline M57.
- M61** **Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi. 1st ed., 2017.** This document provides updated QC tables for the Clinical and Laboratory Standards Institute antifungal susceptibility testing documents M38 and M51.

* CLSI documents are continually reviewed and revised through the CLSI consensus process; therefore, readers should refer to the most current editions.



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