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May 2010

## M51-A

### Method for Antifungal Disk Diffusion Susceptibility Testing of Nondermatophyte Filamentous Fungi; Approved Guideline

This document describes the guidelines for antifungal susceptibility testing by the disk diffusion method of nondermatophyte filamentous fungi (moulds) that cause invasive disease.

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

ISBN 1-56238-725-1  
ISSN 0273-3099

M51-A  
Vol. 30 No. 11  
Replaces M51-P  
Vol. 29 No. 15

## Method for Antifungal Disk Diffusion Susceptibility Testing of Nondermatophyte Filamentous Fungi; Approved Guideline

Volume 30 Number 11

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### Abstract

CLSI broth dilution reference methods are available for susceptibility testing of filamentous fungi (see CLSI document M38)<sup>1</sup> and yeasts (see CLSI documents M27<sup>2</sup> and M44<sup>3</sup>). There still remains, however, a need for an alternative simple, rapid, and cost-effective approach to determine the susceptibility of nondermatophyte filamentous fungi (moulds) to various classes of antifungal agents that would make antifungal susceptibility testing more readily available to clinical microbiology laboratories. The CLSI Subcommittee on Antifungal Susceptibility Testing developed a disk diffusion method for testing filamentous fungi to amphotericin B, caspofungin, itraconazole, posaconazole, and voriconazole.<sup>4</sup> Although clinical breakpoints have not been assigned, epidemiological cutoff values (ECVs) have been developed based on a comparison of zone diameters vs minimal inhibitory concentrations (MICs) or minimal effective concentrations (MECs) using the rate bounding method; control parameters for these agents have also been determined.<sup>4</sup> ECVs are not used as clinical breakpoints, but rather to detect those isolates that are likely to have acquired resistance mechanisms or reduced susceptibility to the tested agent as compared with the wild-type distribution. One significant advantage of this method is that qualitative results can usually be determined after only 16 to 48 hours incubation as opposed to 24 to 72 hours with CLSI document M38.<sup>1</sup> There are more antifungal agents and it is expected that this document will further encourage the development of disk diffusion testing for some of these agents.

Clinical and Laboratory Standards Institute (CLSI). *Method for Antifungal Disk Diffusion Susceptibility Testing of Nondermatophyte Filamentous Fungi; Approved Guideline*. CLSI document M51-A (ISBN 1-56238-725-1). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2010.

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### **Suggested Citation**

CLSI. *Method for Antifungal Disk Diffusion Susceptibility Testing of Nondermatophyte Filamentous Fungi; Approved Guideline*. CLSI document M51-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2010.

### **Previous Edition:**

June 2009

### **Reaffirmed:**

September 2016

### **Archived:**

August 2021

ISBN 1-56238-725-1  
ISSN 0273-3099

**Contents**

Abstract.....	i
Committee Membership.....	iii
Foreword.....	vii
1 Scope.....	1
2 Standard Precautions.....	1
3 Terminology.....	1
3.1 A Note on Terminology.....	1
3.2 Definitions.....	2
3.3 Abbreviations and Acronyms.....	3
4 Selection of Antimicrobial Agent Disks for Routine Testing and Reporting.....	3
4.1 Use of Nonproprietary or Generic Names.....	3
4.2 Number of Agents Tested.....	3
4.3 Suggested Guidelines for Selective Reporting.....	3
5 Equipment/Materials.....	4
6 Test Procedures.....	4
6.1 Nonsupplemented Mueller-Hinton Agar Medium (see Appendix B).....	4
7 Procedure for Performing the Disk Diffusion Test.....	5
7.1 Inoculum Preparation.....	5
7.2 Inoculum Quantitation.....	6
7.3 Inoculation of Test Plates.....	6
7.4 Application of Disks to Inoculated Agar Plates.....	7
7.5 Reading Plates and Interpreting Results.....	7
8 Interpretation of Disks Diffusion Test Results.....	7
8.1 Zone Diameter Epidemiological Cutoff Values.....	7
9 Quality Control Procedures.....	8
9.1 Purpose.....	8
9.2 Standard Reference Strains for Control.....	8
9.3 Storing Reference Strains.....	8
9.4 Zone Diameter Reference Control Limits.....	9
9.5 Frequency of Control Testing.....	9
9.6 Corrective Action.....	10
9.7 Reporting Patient Results When Out-of-Range Results Occur.....	11
10 Limitations of Disk Diffusion Method.....	11
10.1 Application to Various Organism Groups.....	11
10.2 Verification of Patient Results.....	12
References.....	13
Appendix A. Performance of Nonsupplemented Mueller-Hinton Agar.....	15

Appendix B. Preparation of Nonsupplemented Mueller-Hinton Agar .....16

Appendix C. McFarland 0.5 Barium Sulfate Turbidity Standard .....17

Appendix D. Quality Control Protocol Flow Charts.....18

Summary of Delegate Comments and Subcommittee Responses.....20

The Quality Management System Approach .....28

Related CLSI Reference Materials .....29

Sample

## Foreword

Due to the increased incidence of systemic fungal infections and the number of antifungal agents, antifungal susceptibility testing has gained greater recognition. Broth dilution reference methods are now available for susceptibility testing of filamentous fungi (moulds) (see CLSI document M38).<sup>1,5-11</sup> There still remains a need for alternative, simple, rapid, and cost-effective approaches to determine the antifungal susceptibility of these fungi. Disk diffusion methodology has served as an example for yeast testing. A collaborative study has identified parameters for testing the susceptibilities of filamentous fungi to five antifungal agents (amphotericin B, caspofungin, itraconazole, posaconazole, and voriconazole) by the disk diffusion method.<sup>4</sup> This method often provides qualitative results 8 to 24 hours sooner than the standard CLSI document M38<sup>1</sup> method. In addition, the use of nonsupplemented Mueller-Hinton agar in lieu of supplemented Mueller-Hinton agar should make antifungal susceptibility testing more readily available to clinical laboratories at a reduced cost. Although clinical breakpoints have not been assigned, tentative epidemiological cutoff values (ECVs) have been developed, based on a comparison of zone diameters vs minimal inhibitory concentrations (MICs) or minimal effective concentrations (MECs) using the rate bounding method.<sup>4</sup> The ECVs are used to detect those isolates with reduced susceptibility to the tested agent as compared with the wild-type distribution. ECVs are not used as clinical breakpoints, but rather to detect those isolates that are likely to have acquired resistance mechanisms.

## Key Words

Antifungal, antimicrobial, disk, disk diffusion, Kirby-Bauer method, susceptibility testing

## Method for Antifungal Disk Diffusion Susceptibility Testing of Nondermatophyte Filamentous Fungi; Approved Guideline

### 1 Scope

With a need to make antifungal susceptibility testing more readily available to the clinical laboratory, this CLSI document provides an established method for disk diffusion testing of moulds, zone interpretive criteria, and recommended control ranges for amphotericin B, caspofungin, itraconazole, posaconazole, and voriconazole.

The method described in this document is intended for testing moulds that cause invasive disease (*Alternaria* spp., *Aspergillus* spp., *Bipolaris* spp., *Fusarium* spp., *Paecilomyces* spp., *Rhizopus* *bryzae* [*R. arrhizus*] and other mucoraceous [zygomycetes] mould species, the *Pseudallescheria boydii* species complex, and *Scedosporium prolificans*).<sup>4</sup> This method does not currently encompass the yeast or mould form of endemic dimorphic fungi or the dermatophytes.

The method described herein must be followed exactly to obtain reproducible results. When new problems are recognized or improvements in these criteria are developed, changes will be incorporated into future editions of this guideline and also distributed in periodic informational supplements.

This guideline is intended for use by, but not limited to, health care, academic, government, industry, or independent research organizations that perform antifungal susceptibility testing of filamentous fungi.

### 2 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 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 blood-borne pathogens. Standard and universal precaution guidelines are available from the US Centers for Disease Control and Prevention.<sup>12</sup> 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.<sup>13</sup>

### 3 Terminology

#### 3.1 A Note on Terminology

CLSI, as a global leader in standardization, is firmly committed to achieving global harmonization wherever 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 the United States, Europe, and elsewhere; that these differences are reflected in CLSI, International Organization for Standardization (ISO), and European Committee for Standardization (CEN) documents; and that legally required use of terms, regional usage, and different consensus timelines are all important considerations in the harmonization process. In light of this, CLSI’s consensus process for development and revision of standards and guidelines focuses on harmonization of terms to facilitate the global application of standards and guidelines.

Of particular note in CLSI document M51-A are two terms whereby CLSI intends to eliminate confusion over time through its commitment to harmonization. For the most part, in this guideline, the term

“accuracy,” in its metrological sense, refers to the closeness of the agreement between the result of a single measurement and a true value of a measurand, thus comprising both random and systematic effects. The term “trueness,” usually used to replace the term “accuracy” when referring to the closeness of agreement does not apply in M51-A because it refers to the closeness of agreement between the average value obtained from a large series of test results and an accepted reference value.

### 3.2 Definitions

**accuracy (measurement)** – closeness of agreement between a measured quantity value and a true quantity value of a measurand (ISO/IEC Guide 99).<sup>14</sup>

**clinical breakpoint** – **1)** a classification based on an *in vitro* response of an organism to an antimicrobial agent at levels corresponding to blood or tissue levels attainable with usually prescribed doses; **2) susceptible clinical breakpoint** – a category that implies that an infection due to the isolate may be appropriately treated with the dosage of an antimicrobial agent recommended for that type of infection and infecting species, unless otherwise contraindicated; **3) intermediate clinical breakpoint** – a category that includes isolates with antimicrobial agent minimal inhibitory concentrations (MICs) or minimal effective concentrations (MECs) that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates; **4) resistant clinical breakpoint** – a category that includes resistant isolates that are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules or where clinical efficacy has not been reliable in treatment studies.

**epidemiological cutoff value (ECV)** – the ECV for each agent is the value obtained by considering the wild-type distribution, the modal MIC/MEC for each distribution, and the inherent variability of the test. Usually, the ECV encompasses at least 95% of isolates in the wild-type distribution<sup>15</sup>; **NOTE:** Organisms with acquired resistance mechanisms may be included among those for which the MICs/MECs are higher than the ECV (for disk testing, those with acquired resistance mechanisms would show a zone diameter smaller than the ECV).

**minimal effective concentration (MEC)** – the lowest concentration of an antimicrobial agent that leads to the growth of small, rounded, compact hyphal forms as compared to the hyphal growth seen in the growth control well; **NOTE:** This terminology is currently used only with respect to testing of the echinocandin antifungal agents.

**minimal inhibitory concentration (MIC)** – the lowest concentration of an antimicrobial agent that causes a specified reduction in visible growth of a microorganism in an agar or broth dilution susceptibility test.

**modal MIC/MEC** – the most frequent MIC or MEC found within an MIC or MEC distribution.

**precision (measurement)** – closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions (ISO/IEC Guide 99).<sup>14</sup>

**quality control** – part of quality management focused on fulfilling quality requirements (ISO 9000)<sup>16</sup>; **NOTE:** This includes operational techniques and activities used to fulfill these requirements.

**reproducibility (measurement)** – measurement precision (closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions) under reproducibility conditions of measurement (condition of measurement, out of a set of conditions that includes different locations, operators, measuring systems, and replicate measurements on the same or similar objects) (ISO/IEC Guide 99).<sup>14</sup>

## The Quality Management System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management system approach in the development of standards and guidelines, which facilitates project management; defines a document structure via a template; and provides a process to identify needed documents. The approach is based on the model presented in CLSI document HS01—A *Quality Management System Model for Health Care*. The quality management system 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 as follows:

Documents and Records Organization	Equipment Purchasing and Inventory Process Control	Information Management Occurrence Management Assessments—External and Internal	Process Improvement Customer Service Facilities and Safety
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M51-A addresses the QSEs indicated by an “X.” For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

Documents and Records	Organization	Personnel	Equipment	Purchasing and Inventory	Process Control	Information Management	Occurrence Management	Assessments—External and Internal	Process Improvement	Customer Service	Facilities and Safety
ILA21		ILA21	ILA21		X ILA21 M02 M27  M38 M44	ILA21		ILA21	ILA21		ILA21  M29

Adapted from CLSI document HS01—A *Quality Management System Model for Health Care*.

### Path of Workflow

A path of workflow is the description of the necessary steps to deliver the particular product or service that the organization or entity provides. For example, CLSI document GP26—*Application of a Quality Management System Model for Laboratory Services* defines a clinical laboratory path of workflow, which consists of three sequential processes: preexamination, examination, and postexamination. All clinical laboratories follow these processes to deliver the laboratory’s services, namely quality laboratory information.

M51-A addresses the clinical laboratory path of workflow steps indicated by an “X.” For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

Preexamination				Examination			Postexamination	
Examination ordering	Sample collection	Sample transport	Sample receipt/processing	Examination	Results review and follow-up	Interpretation	Results reporting and archiving	Sample management
				X M02 M27 M38	X M02 M27 M38 M44	X M02 M27 M38 M44	X M02 M27 M38 M44	X  M27 M38

Adapted from CLSI document HS01—A *Quality Management System Model for Health Care*.

## Related CLSI Reference Materials\*

- I/LA21-A2** **Clinical Evaluation of Immunoassays; Approved Guideline—Second Edition (2008).** This document addresses the need for clinical evaluation of new immunoassays and new applications of existing assays, as well as multiple assay formats and their uses. As a guide to designing and executing a clinical evaluation, this document will aid developers of “in-house” assays for institutional use, developers of assays used for monitoring pharmacologic effects of new drugs or biologics, and clinical and regulatory personnel responsible for commercializing products.
- M02-A10** **Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Tenth Edition (2009).** This document contains the current CLSI-recommended methods for disk susceptibility testing, criteria for quality control testing, and updated tables for interpretive zone diameters.
- M23-A3** **Development of *In Vitro* Susceptibility Testing Criteria and Quality Control Parameters; Approved Guideline—Third Edition (2008).** This document addresses the required and recommended data needed for the selection of appropriate interpretive criteria and quality control ranges for antimicrobial agents.
- M27-A3** **Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard—Third Edition (2008).** This document addresses the selection and preparation of antifungal agents; implementation and interpretation of test procedures; and quality control requirements for susceptibility testing of yeasts that cause invasive fungal infections.
- M29-A3** **Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Third Edition (2005).** 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.
- M38-A2** **Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard—Second Edition (2008).** This document addresses the selection of antifungal agents, preparation of antifungal stock solutions and dilutions for testing implementation and interpretation of test procedures, and quality control requirements for susceptibility testing of filamentous fungi (moulds) that cause invasive and cutaneous fungal infections.
- M44-A2** **Methods for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guideline—Second Edition (2009).** This document provides newly established methodology for disk diffusion testing of *Candida* spp., criteria for quality control testing, and interpretive criteria.
- M51-S1** **Performance Standards for Antifungal Disk Diffusion Susceptibility Testing of Nondermatophyte Filamentous Fungi; Informational Supplement (2010).** These supplemental tables provide zone diameter reference limits for CLSI document M51-A.

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

# Sample



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