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# 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.

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

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Health care professionals in all specialties are urged to volunteer for participation in CLSI projects. Please contact us at customerservice@clsi.org or +610.688.0100 for additional information on committee participation.

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## Abstract

CLSI broth dilution reference methods are available for susceptibility testing of filamentous fungi (see CLSI document M38)[1] and yeasts (see CLSI documents M27[2] and M44[3]). 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.[4] 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.[4] 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.[1] 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.

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## Contents

## 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).[1,5-11] 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.[4] This method often provides qualitative results 8 to 24 hours sooner than the standard CLSI document M38[1] 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.[4] 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

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## 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 oryzae* [*R. arrhizus*] and other mucoraceous [zygomycetes] mould species, the *Pseudallescheria boydii* species complex, and *Scedosporium prolificans)*.[4] 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.[12] 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.[13]

## 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