



M44-A2

Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guideline—Second Edition

This document provides newly established methodology for disk diffusion testing of *Candida* spp., criteria for quality control testing, and interpretive criteria.

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

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Abstract

Clinical and Laboratory Standards Institute (CLSI) broth dilution reference methods are available for susceptibility testing of yeasts (see CLSI document M27—*Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts*) and moulds (see CLSI document M38—*Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi*). There still remains, however, a need for an alternative simple, rapid, and cost-effective approach to determine susceptibility of fungal organisms to various classes of antifungal agents that would make antifungal susceptibility testing more readily available to the clinical microbiology laboratory. The CLSI Subcommittee on Antifungal Susceptibility Testing has therefore developed a disk diffusion method for testing yeasts. CLSI document M44-A2—*Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guideline—Second Edition* provides approved zone interpretive criteria (breakpoints) for *Candida* species for caspofungin, fluconazole, and voriconazole after 20 to 24 hours incubation, as well as quality control parameters for caspofungin, fluconazole, posaconazole, and voriconazole. There are currently more than 10 systemically active antifungal agents, and it is expected that this document will further encourage the development of disk diffusion testing for at least some of these additional agents and genera.

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Contents

Abstract.....	i
Committee Membership.....	iii
Foreword.....	vii
1 Scope.....	1
2 Standard Precautions.....	1
3 Terminology.....	1
3.1 Note on Terminology.....	1
3.2 Definitions.....	2
3.3 Abbreviations and Acronyms.....	2
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.....	3
6 Reagents for the Disk Diffusion Test.....	3
6.1 Mueller-Hinton Agar + 2% Glucose and 0.5 µg/mL Methylene Blue Dye (GMB) Medium (see Appendix A).....	3
6.2 Storage of Antimicrobial Disks.....	4
6.3 Turbidity Standard for Inoculum Preparation.....	5
7 Procedure for Performing the Disk Diffusion Test.....	5
7.1 Inoculum Preparation: Direct Colony Suspension Method.....	5
7.2 Inoculation of Test Plates.....	5
7.3 Application of Disks to Inoculated Agar Plates.....	6
7.4 Reading Plates and Interpreting Results.....	6
8 Interpretation of Disk Diffusion Test Results.....	6
8.1 Zone Diameter Interpretive Standards.....	6
8.2 Interpretive Categories.....	6
8.3 Zone Diameter Interpretive Criteria.....	7
9 Quality Control Procedures.....	7
9.1 Purpose.....	7
9.2 Reference Strains for Quality Control.....	8
9.3 Storing Quality Control Strains.....	8
9.4 Zone Diameter Quality Control Limits.....	8
9.5 Frequency of Quality Control Testing.....	8
9.6 Corrective Action.....	9
9.7 Reporting Patient Results When Out-of-Range Tests Occur.....	10
10 Limitations of Disk Diffusion Methods.....	11
10.1 Application to Various Organism Groups.....	11
10.2 Verification of Patient Results.....	11

Contents (Continued)

References..... 12

Appendix A. Preparation of Mueller-Hinton Agar + 2% Glucose and 0.5 µg/mL Methylene Blue Dye..... 13

Appendix B. McFarland 0.5 Barium Sulfate Turbidity Standard 15

Appendix C. Quality Control Protocol Flow Charts..... 16

Summary of Delegate Comments and Subcommittee Responses..... 18

The Quality Management System Approach 22

Related CLSI Reference Materials 23

SAMPLE

Foreword

Owing to the increased incidence of systemic fungal infections and number of antifungal agents available for systemic administration, antifungal susceptibility testing has gained greater recognition. Today, antifungal susceptibility testing has come of age in guiding physicians in the selection of antifungal therapy. Broth macrodilution and microdilution reference methods are now available for susceptibility testing of both yeasts (see CLSI document M27¹) and moulds (see CLSI document M38²). To make antifungal susceptibility testing more readily available to clinical microbiology laboratories, there still remains a need for alternative, simple, rapid, and cost-effective approaches. Disk diffusion testing has served as such an example for antibacterial testing (see CLSI document M02³), and therefore, the CLSI Subcommittee on Antifungal Susceptibility Tests has developed recommendations for disk diffusion testing for antifungal agents.

A disk diffusion method for testing yeasts has been developed. At present, this method is validated only for *Candida* spp. tested vs various azoles and echinocandins. This method provides qualitative results after 20 to 24 hours incubation. In addition, the use of supplemented Mueller-Hinton agar in lieu of RPMI 1640 medium should make antifungal susceptibility testing more readily available to at least some clinical laboratories, and at reduced cost. Zone interpretive criteria (breakpoints) for caspofungin, fluconazole, and voriconazole, and quality control parameters for caspofungin, fluconazole, posaconazole, and voriconazole have been established according to standard CLSI procedures. CLSI expects that this document will encourage the development of disk diffusion testing for other antifungal agents and fungal genera.

Key Words

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

SAMPLE

Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guideline—Second Edition

1 Scope

With a need to make antifungal susceptibility testing more readily available to the clinical laboratory, CLSI document M44-A2 provides an established methodology for disk diffusion testing of *Candida* spp.; zone interpretive criteria for caspofungin, fluconazole, and voriconazole; and recommended quality control ranges for caspofungin, fluconazole, posaconazole, and voriconazole.

The method described in this document is intended for testing *Candida* spp. This method does not currently encompass any other genera and has not been used in studies of the yeast form of dimorphic fungi, such as *Blastomyces dermatitidis* or *Histoplasma capsulatum*. Moreover, testing of filamentous fungi (ie, moulds) is not addressed in the current procedure.

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 yeasts.

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 the major features of “universal precautions and body substance isolation” practices. Standard precautions cover the transmission of all 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.⁴ For specific precautions for preventing the laboratory transmission of all infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all infectious diseases, refer to CLSI document M29.⁵

3 Terminology

3.1 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 focuses on harmonization of terms to facilitate the global application of standards.

Of particular note in CLSI document M44-A2 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 agreement between a measured

quantity value and a true quantity 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 M44, because it refers to the closeness of agreement between the average of an infinite number of replicate measured quantity values and a reference quantity 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).⁶

antibiogram – overall profile of antimicrobial susceptibility results of a microbial species to a battery of antimicrobial agents.

antimicrobial susceptibility test interpretive category – 1) a classification based on an *in vitro* response of an organism to an antimicrobial agent at levels of that agent corresponding to blood or tissue levels attainable with usually prescribed doses of that agent; **2) susceptible antimicrobial susceptibility test interpretive category** – 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) susceptible-dose dependent (S-DD) antimicrobial susceptibility test interpretive category** – a category that includes isolates with antimicrobial agent minimal inhibitory concentrations (MICs) that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates; **4) intermediate (I) antimicrobial susceptibility test interpretive category** – a category that includes isolates with antimicrobial agent MICs that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates and/or available data do not permit them to be clearly categorized as either “susceptible” or “resistant”; **NOTE:** This category also includes a buffer zone, which should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations; **5) resistant antimicrobial susceptibility test interpretive category** – 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; **6) nonsusceptible (NS) test interpretive category** – a category used for organisms that currently have only a susceptible interpretive category, but not susceptible-dose dependent, intermediate, or resistant interpretive categories (ie, susceptible-only interpretive category); **NOTE:** This category is often given to new antimicrobial agents for which no resistant isolates have yet been encountered.

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).⁶

quality control – part of quality management focused on fulfilling quality requirements (ISO 9000)⁷; **NOTE:** This includes operational techniques and activities used to fulfill these requirements.

repeatability (measurement) – measurement precision under a set of repeatability conditions of measurement (ISO/IEC Guide 99).⁶

repeatability condition (of measurement) – condition of measurement, out of a set of conditions that includes the same measurement procedure, same operators, same measuring system, same operating conditions and same location, and replicate measurements on the same or similar objects over a short period of time (ISO/IEC Guide 99).⁶

3.3 Abbreviations and Acronyms

GMB	2% glucose and 0.5 µg/mL methylene blue dye
MIC	minimal inhibitory concentration

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 that 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 Personnel	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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M44-A2 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
M07					X M02 M06 M07 M23 M27 M29 M38						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.

M44-A2 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
				M02 M07 M27 M38	X M02 M07 M27 M38	X M02 M07 M27 M38	X M02 M07 M27 M38	M27 M38

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

Related CLSI Reference Materials*

- M02-A10** **Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Tenth Edition (2009).** This document contains the current Clinical and Laboratory Standards Institute–recommended methods for disk susceptibility testing, criteria for quality control testing, and updated tables for interpretive zone diameters.
- M06-A2** **Protocols for Evaluating Dehydrated Mueller-Hinton Agar; Approved Standard—Second Edition (2006).** This document provides procedures for evaluating production lots of dehydrated Mueller-Hinton agar, and for developing and applying reference media.
- M07-A8** **Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Eighth Edition (2009).** This document addresses reference methods for the determination of minimal inhibitory concentrations (MICs) of aerobic bacteria by broth macrodilution, broth microdilution, and agar dilution.
- 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 standard 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.

* 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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