CLINICAL AND LABORATORY STANDARDS INSTITUTE®

3rd Edition

M44

Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts

This guideline provides an established methodology for disk diffusion testing of *Candida* spp., along with recommendations for results interpretation and quality control testing.

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

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Clinical and Laboratory Standards Institute
950 West Valley Road, Suite 2500
Wayne, PA 19087 USA
P: +1.610.688.0100
F: +1.610.688.0700
www.clsi.org
standard@clsi.org

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Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts

Gary W. Procop, MD, MS
Barbara D. Alexander, MD, MHS
Philippe J. Dufresne, PhD, RMCCM
Jeff Fuller, PhD, FCCM, D(ABMM)
Mahmoud A. Ghannoum, PhD, FIDSA, MBA
Kimberly E. Hanson, MD, MHS
Denise Holliday, MT(ASCP)
Nicole M. Holliday, BA
Luis Ostrosky-Zeichner, MD, FACP, FIDSA, FSHEA, CMQ
Audrey N. Schuetz, MD, MPH, D(ABMM)
Nathan P. Wiederhold, PharmD
Adrian M. Zelazny, PhD, D(ABMM)

Abstract

Clinical and Laboratory Standards Institute guideline M44—*Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts* provides an approved method for determining zone diameter breakpoints for select *Candida* spp. with antifungal agents after 24 hours incubation, as well as quality control parameters for the same agents. This guideline fulfills the need for an alternative to the broth microdilution testing procedure (see CLSI document M27[1]) and describes a simple, rapid, and cost-effective approach for determining fungal organisms' susceptibility to various classes of antifungal agents. It also makes antifungal susceptibility testing more readily available to the medical microbiology laboratory and encourages the development of disk diffusion testing for newly discovered antifungal agents.

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Committee Membership

Consensus Council

Dennis J. Ernst, MT(ASCP), NCPT(NCCT)
Chairholder
Center for Phlebotomy Education
USA

Mary Lou Gantzer, PhD, FACB
Vice-Chairholder
USA

J. Rex Astles, PhD, FACB, DABCC
Centers for Disease Control and Prevention
USA

Lucia M. Berte, MA, MT(ASCP)SBB, DLM, CQA(ASQ)CMQ/OE
Laboratories Made Better!
USA

Karen W. Dyer, MT(ASCP), DLM
Centers for Medicare & Medicaid Services
USA

Thomas R. Fritsche, MD, PhD, FCAP, FIDSA
Marshfield Clinic
USA

Loralie J. Langman, PhD, DABCC, FACB, F-ABFT
Mayo Clinic
USA

James R. Petisce, PhD
BD Diagnostic Systems
USA

Andrew Quintenz
Bio-Rad Laboratories, Inc.
USA

Robert Rej, PhD
New York State Department of Health – Wadsworth Center
USA

Zivana Tezak, PhD
FDA Center for Devices and Radiological Health
USA

Subcommittee on Antifungal Susceptibility Tests

Gary W. Procop, MD, MS
Chairholder
Cleveland Clinic
USA

Barbara D. Alexander, MD, MHS
Vice-Chairholder
Duke University Medical Center
USA

Camille Hamula, PhD, D(ABMM)
Committee Secretary
Mount Sinai Health System
USA

Philippe J. Dufresne, PhD, RMCCM
Institut national de santé publique du Québec
Canada

Jeff Fuller, PhD, FCCM, D(ABMM)
London Health Sciences Centre
Canada

Mahmoud A. Ghannoum, PhD, FIDSA, MBA
Case Western Reserve University
USA

Kimberly E. Hanson, MD, MHS
ARUP at University Hospital
USA

Denise Holliday, MT(ASCP)
BD Diagnostic Systems
USA

Nicole M. Holliday, BA
Thermo Fisher Scientific
USA

Luis Ostrosky-Zeichner, MD, FACP, FIDSA, FSHEA, CMQ
McGovern Medical School
USA

Audrey N. Schuetz, MD, MPH, D(ABMM)
Mayo Clinic
USA

Nathan P. Wiederhold, PharmD
University of Texas Health Science Center at San Antonio
USA

Adrian M. Zelazny, PhD
USA

Staff

Clinical and Laboratory Standards Institute
USA

Marcy L. Hackenbrack, MCM, M(ASCP)
Project Manager

Megan L. Tertel, MA, ELS
Editorial Manager

Catherine E.M. Jenkins
Editor

Kristy L. Leirer, MS
Editor

Laura Martin
Editor

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Expert Panel on Microbiology

Richard B. Thomson Jr., PhD, D(ABMM), FAAM
Chairholder
Evanston Hospital, NorthShore University HealthSystem
USA

Jean B. Patel, PhD, D(ABMM)
Vice-Chairholder
Centers for Disease Control and Prevention
USA

Kevin Alby, PhD, D(ABMM)
University of Pennsylvania Health System
USA

Lynette Y. Berkeley, PhD, MT(ASCP)
FDA Center for Drug Evaluation and Research
USA

Carey-Ann Burnham, PhD, D(ABMM)
Washington University School of Medicine
USA

Karissa Culbreath, PhD, D(ABMM)
University of New Mexico Department of Pathology
USA

German Esparza, BSc
Proasecal SAS
Colombia

Margie Morgan, PhD, D(ABMM)
Cedars-Sinai Medical Center
USA

Mark G. Papich, MS, DVM
North Carolina State University, College of Veterinary Medicine
USA

David H. Pincus, MS, RM/SM(NRCM), SM(ASCP)
bioMérieux, Inc.
USA

Audrey N. Schuetz, MD, MPH, D(ABMM)
Mayo Clinic
USA

Ribhi M. Shawar, PhD, D(ABMM)
FDA Center for Devices and Radiological Health
USA

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Kimberly E. Hanson, MD, MHS
ARUP at University Hospital
USA

Gary W. Procop, MD, MS
Cleveland Clinic
USA

Contents

Foreword

Because of the increased incidence of systemic fungal infections and increasing number of antifungal agents available for systemic administration, antifungal susceptibility testing has become more common. Today, antifungal susceptibility testing is an important tool for guiding physicians in selecting antifungal therapy. Broth macrodilution and microdilution reference methods are now available for yeast (see CLSI document M27[1]) and mould (see CLSI document M38[2]) susceptibility testing. For antifungal susceptibility testing to become more readily available to medical microbiology laboratories, simple, rapid, and cost-effective alternative approaches are needed. The disk diffusion method used for antibacterial testing (see CLSI document M02[3]) has provided the basis for a simple method for susceptibility testing of yeasts. Therefore, the CLSI Subcommittee on Antifungal Susceptibility Tests has developed a disk diffusion method for testing susceptibility of yeasts to antifungal agents.

Currently, this method is validated only for select *Candida* spp. tested vs various azoles and echinocandins. This method provides qualitative results after 24 hours incubation. Using supplemented Mueller-Hinton agar in lieu of RPMI 1640 medium makes antifungal susceptibility testing more readily available and less costly to some medical laboratories. Zone diameter breakpoints for caspofungin, micafungin, fluconazole, and voriconazole and quality control parameters for caspofungin, micafungin, fluconazole, posaconazole, and voriconazole have been established according to standard CLSI procedures. CLSI expects that this guideline will encourage disk diffusion testing development for other antifungal agents and fungal genera.

Overview of Changes

This guideline replaces the previous edition of the approved guideline, M44-A2, published in 2009. Several changes were made in this edition, including:

- Reorganized to fit the CLSI quality management system and path of workflow format
- Revised definitions for interpretive categories to align with other CLSI susceptibility testing documents
- Added disk diffusion standards for micafungin
- Described the specific *Candida* spp. for which there are zone diameter breakpoints and interpretive categories
- Updated yeast nomenclature
- Updated references to the previous informational supplement (M44-S3) to reflect CLSI document M60,[4] the new supplement for broth dilution and disk diffusion yeast susceptibility testing

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

Key Words

Antifungal agent, antimicrobial agent, *Candida* spp., disk, disk diffusion, Kirby-Bauer method, susceptibility testing, yeast

Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts

Chapter 1: Introduction

This chapter includes:

- Guideline's scope and applicable exclusions
- 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 guideline
- Abbreviations and acronyms used in the guideline

1.1 Scope

This guideline provides an established methodology for disk diffusion testing of select *Candida* spp. For zone diameter breakpoints, interpretive categories, and recommended QC ranges for caspofungin, micafungin, fluconazole, and voriconazole, refer to CLSI document M60.[4]

The method described is intended for testing select *Candida* spp. This method is not currently applicable to any other genera and has not been used in studies of the yeast form of dimorphic fungi (eg, *Blastomyces dermatitidis* or *Histoplasma capsulatum*). Moreover, testing of filamentous fungi (ie, moulds) is not covered in the current procedure.

The method described in this guideline 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 M44 and new breakpoint information will be distributed in periodic informational supplements (see CLSI document M60[4]).

This guideline is intended for use by, among others, health care, academic, government, industry, or independent research organizations that perform antifungal susceptibility testing of yeasts.

1.2 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 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.[5] 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.[6]