CLINICAL AND LABORATORY STANDARDS INSTITUTE®

3rd Edition

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.

Clinical and Laboratory Standards Institute

Setting the standard for quality in medical laboratory testing around the world.

The Clinical and Laboratory Standards Institute (CLSI) is a not-for-profit membership organization that brings together the varied perspectives and expertise of the worldwide laboratory community for the advancement of a common cause: to foster excellence in laboratory medicine by developing and implementing medical laboratory standards and guidelines that help laboratories fulfill their responsibilities with efficiency, effectiveness, and global applicability.

Consensus Process

Consensus—the substantial agreement by materially affected, competent, and interested parties—is core to the development of all CLSI documents. It does not always connote unanimous agreement but does mean that the participants in the development of a consensus document have considered and resolved all relevant objections and accept the resulting agreement.

Commenting on Documents

CLSI documents undergo periodic evaluation and modification to keep pace with advances in technologies, procedures, methods, and protocols affecting the laboratory or health care.

CLSI's consensus process depends on experts who volunteer to serve as contributing authors and/or as participants in the reviewing and commenting process. At the end of each comment period, the committee that developed the document is obligated to review all comments, respond in writing to all substantive comments, and revise the draft document as appropriate.

Comments on published CLSI documents are equally essential and may be submitted by anyone, at any time, on any document. All comments are managed according to the consensus process by a committee of experts.

Appeal Process

When it is believed that an objection has not been adequately considered and responded to, the process for appeal, documented in the CLSI *Standards Development Policies and Processes,* is followed.

All comments and responses submitted on draft and published documents are retained on file at CLSI and are available upon request.

Get Involved—Volunteer!

Do you use CLSI documents in your workplace? Do you see room for improvement? Would you like to get involved in the revision process? Or maybe you see a need to develop a new document for an emerging technology? CLSI wants to hear from you. We are always looking for volunteers. By donating your time and talents to improve the standards that affect your own work, you will play an active role in improving public health across the globe.

For additional information on committee participation or to submit comments, contact CLSI.

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

M38, 3rd ed.
November 2017
Replaces M38-A2

Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi

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

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.

Clinical and Laboratory Standards Institute (CLSI). *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi*. 3rd ed. CLSI standard M38 (ISBN 1-56238-830-4 [Print]; ISBN 1-56238-831-2 [Electronic]). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2017.

Suggested Citation

CLSI. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi*. 3rd ed. CLSI standard M38. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.

Previous Editions:
November 1998, August 2002, April 2008

ISBN 1-56238-830-4 (Print)
ISBN 1-56238-831-2 (Electronic)
ISSN 1558-6502 (Print)
ISSN 2162-2914 (Electronic) Volume 37, Number 15

Committee Membership

Consensus Council

Carl D. Mottram, RRT, RPFT, FAARC
Chairholder
Mayo Clinic
USA

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

Mary Lou Gantzer, PhD, FACB
BioCore Diagnostics
USA

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

Ross J. Molinaro, PhD, MLS(ASCP)CM, DABCC, FACB
Siemens Healthcare Diagnostics, Inc.
USA

Joseph Passarelli
Roche Diagnostics Corporation
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

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

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

Philippe 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, EMBA, FIDSA
Case Western Reserve University
USA

Kimberly E. Hanson, MD, MHS
University of Utah and ARUP Laboratories
USA

Denise Holliday, MT(ASCP)
BD Diagnostic Systems
USA

Nicole M. Holliday, BA
Thermo Fisher Scientific
USA

Luis Ostrosky-Zeichner, MD, FACP, FIDSA, FSHEA
Memorial Hermann Healthcare System
USA

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

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

Adrian M. Zelazny, PhD, D(ABMM)
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

Acknowledgment for the Expert Panel on Microbiology

CLSI, the Consensus Council, and the Subcommittee on Antifungal Susceptibility Tests gratefully acknowledge the Expert Panel on Microbiology for serving as technical advisors and subject matter experts during the development of this standard.

Expert Panel on Microbiology

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

Mary Jane Ferraro, PhD, MPH
Vice-Chairholder
Massachusetts General Hospital
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

German Esparza, BSc
Proasecal LTD
Colombia

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

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

Barbara L. Zimmer, PhD
Beckman Coulter - West Sacramento
USA

Acknowledgment

CLSI, the Consensus Council, and the Subcommittee on Antifungal Susceptibility Tests gratefully acknowledge the following volunteers for their important contributions to the development of this standard:

Ana Espinel-Ingroff, PhD, MS
Virginia Commonwealth University Health System
USA

Philippe Dufresne, PhD (RMCCM)
Institut national de santé publique du Québec
Canada

Acknowledgment

CLSI, the Consensus Council, and the Subcommittee on Antifungal Susceptibility Tests gratefully acknowledge the following former subcommittee members for their review of this standard during development:

Sharon K. Cullen, BS, PMP, RAC
Beckman Coulter - West Sacramento
USA

Shawn R. Lockhart, PhD, D(ABMM)
Centers for Disease Control and Prevention
USA

David S. Perlin, PhD
New Jersey Medical School-UMDNJ
USA

Dee Shortridge, PhD
JMI Laboratories
USA

Nancy L. Wengenack, PhD, D(ABMM)
Mayo Clinic
USA

Contents

Contents (Continued)

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 M57[11] and M59[12]).[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 M57[11] 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 M57[11] and M59[12]
- **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 M61[18]

  - 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 M61[18]

  - 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 M27[20]

  - Harmonized dilution schemes for dermatophyte and nondermatophyte isolates with those in CLSI document M27[20] 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 M27[20] 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 M61[18] 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.