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M38-A2

Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard—Second Edition

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.

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

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Abstract

Clinical and Laboratory Standards Institute document M38-A2—*Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard—Second Edition* describes a method for testing the susceptibility of filamentous fungi (moulds) that cause invasive (*Aspergillus* spp., *Fusarium* spp., *Rhizopus oryzae (R. arrhizus*), *Pseudallescheria boydii [Scedosporium apiospermum], S. prolificans*, *Sporothrix schenckii*, and other opportunistic pathogenic moulds) and cutaneous (dermatophyte, *Trichophyton*, *Microsporum*, and *Epidermophyton* spp.) fungal infections to antifungal agents. Selection of antifungal agents; preparation of antifungal stock solutions and dilutions for testing, implementation, and interpretation of test procedures; and the purpose and implementation of quality control procedures are discussed. A careful examination of the responsibilities of the manufacturer and the user in quality control is also presented.

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Foreword

With the increased incidence of systemic fungal infections and the growing number of antifungal agents, laboratory methods to guide the selection of antifungal therapy have gained greater attention. The CLSI Area Committee on Microbiology formed the Subcommittee on Antifungal Susceptibility Testing, and data for testing filamentous fungi were collected in a series of collaborative studies. As a result, CLSI document M27[1] was published with the establishment of quality control MIC ranges and the development of breakpoints.

Based on these achievements, the subcommittee concluded that it would be useful to work toward a reproducible reference testing procedure for the antifungal susceptibility testing of filamentous fungi (moulds). A working group on filamentous fungi was formed and charged with the responsibility of carrying out studies to collect data and to refine the methodology to perform susceptibility testing of these fungal species. As a result of several collaborative studies, agreement within the subcommittee was achieved regarding testing conditions for the nondermatophyte moulds that included inoculum preparation and inoculum size, incubation time and temperature, medium formulation, and criteria for MIC determination.[2-5] This consensus method was published in 2002 as M38-A.

In M38-A2, supplemental material (QC data for mould isolates as well as echinocandin testing guidelines) has been incorporated.[6-9] In addition, methods for testing dermatophyte moulds are provided, based on a series of consensus studies.[10,11]

Because of its suitability for antifungal susceptibility testing of yeasts, synthetic RPMI-1640 medium was the test medium that the subcommittee evaluated as the potential reference medium for moulds including the dermatophytes.[2,3,10,12] The subcommittee has evaluated other media formulations, but the standard RPMI medium facilitated more consistent identification of itraconazole resistance in *Aspergillus* spp. in eight laboratories.[5] Drug stock solution preparation and dilution previously developed for antifungal testing of yeasts procedures (CLSI document M27)[1] also were adopted.

Key Words

antifungal, broth microdilution, dermatophytes, filamentous fungi or moulds, susceptibility testing

Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard—Second Edition

1 Scope

This document describes a method for testing the susceptibility of filamentous fungi (moulds) that cause invasive (*Aspergillus* spp., *Fusarium* spp., *Rhizopus oryzae* [*R. arrhizus*], *Pseudallescheria boydii* [*Scedosporium apiospermum*], *Sporothrix schenckii,* and other pathogenic moulds) and cutaneous (the dermatophytes *Trichophyton*, *Microsporum,* and *Epidermophyton* spp.) fungal infections to antifungal agents.[2-5,10] Addressed in this document are testing conditions including inoculum preparation and inoculum size, incubation time and temperature, medium formulation, and criteria end-point determination.[7-9] Quality control (QC) reference ranges are also provided.[6,11]

This standard focuses on the fully defined synthetic medium RPMI-1640 for testing of moulds because of the suitability of this test medium for antifungal susceptibility testing of yeasts.[2,3,11,12]

Refer to CLSI document M27[1] for drug stock solution preparation and dilution procedures.

2 Introduction

The method described in this document is intended for testing common filamentous fungi or moulds, including the dermatophytes, which cause invasive and cutaneous infections, respectively. These moulds encompass *Aspergillus* spp., *Fusarium* spp., *Rhizopus* spp., *P. boydii (S. apiospermum), S. prolificans*, the mycelial form of *S. schenckii*, other Zygomycetes and opportunistic monilaceous and dematiaceous moulds,[4,12] as well as the dermatophyte *Trichophyton*, *Microsporum,* and *Epidermophyton* spp.[10] Caution should be used when interpreting the minimal inhibitory concentration (MIC) and minimal effective concentration (MEC) results for any mould/drug combination. The method has not been used in studies of the yeast or mould form of dimorphic fungi, such as *Blastomyces dermatitidis*, *Coccidioides immitis*, *Coccidioides posadasii, Histoplasma capsulatum* variety *capsulatum*, *Penicillium marneffei*, or *S. schenckii*. The method also has not been used in studies of dermatophytes with the echinocandins or nondermatophyte moulds with ciclopirox, griseofulvin, or terbinafine.

This document is a "reference" standard developed through a consensus process to facilitate agreement among laboratories in measuring the susceptibility of moulds to antifungal agents. It is emphasized that the relationship between *in vitro* vs *in vivo* data has only been evaluated in animal models.[12] An important use of a reference method is to provide a standard basis from which other methods can be developed, which also will result in interlaboratory agreement within specified ranges. Such methods might have particular advantages, such as ease of performance, economy, or more rapid results; therefore, their development could be highly desirable. To the extent that any method produces concordant results with this reference method, it would be considered to be in conformity with M38-A2.

3 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.[13] 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 disease, refer to CLSI document M29.[14]

4 Definitions

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

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 (see Appendix A).

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.

quality control (QC) – the operational techniques that are used to ensure accuracy and reproducibility.

5 Antifungal Agents

5.1 Source

Antifungal standards or reference powders can be obtained commercially, directly from the drug manufacturer. Pharmacy stock or other clinical preparations should not be used. Acceptable powders bear a label that states the drug's generic name, its assay potency (usually expressed in micrograms [μg] or International Units per mg of powder), and its expiration date. Store the powders as recommended by the manufacturers, or at −20 °C or below (never in a self-defrosting freezer), in a desiccator, preferably in a vacuum. When the desiccator is removed from the freezer, allow it to come to room temperature before opening (to avoid condensation of water).

5.2 Weighing Antifungal Powders

Assay all antifungal agents for standard units of activity. The assay units can differ widely from the actual weight of the powder and often differ within a drug production lot. Thus, a laboratory must standardize its antifungal solutions based on assays of the lots of antifungal powders used.

Use either of the following formulas to determine the amount of powder or diluent needed for a standard solution:

$$\text{Weight (mg)} = \frac{\text{Volume (mL)} \bullet \text{Concentration (μg/mL)}}{\text{Potency (μg/mg)}} \quad (1)$$

or

$$\text{Volume (mL)} = \frac{\text{Weight (mg)} \bullet \text{Potency (μg/mg)}}{\text{Concentration (μg/mL)}} \quad (2)$$

The antifungal powder should be weighed on an analytical balance that has been calibrated by approved reference weights from a national metrology organization. Usually, it is advisable to accurately weigh a portion of the antifungal agent in excess of that required and to calculate the volume of diluent needed to obtain the concentration desired.

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 the most current edition of CLSI/NCCLS document HS1—*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:

Documents & Records	Equipment	Information Management	Process Improvement
Organization	Purchasing & Inventory	Occurrence Management	Customer Service
Personnel	Process Control	Assessments—External & Internal	Facilities & Safety

M38-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 & Records	Organization	Personnel	Equipment	Purchasing & Inventory	Process Control	Information Management	Occurrence Management	Assessments—External & Internal	Process Improvement	Customer Service	Facilities & Safety
M7					X M2 M7 M11 M23 M24 M27 M29						M29

Adapted from CLSI/NCCLS document HS1—*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/NCCLS 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.

M38-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
			M24	X M24 M27	X M2 M7 M11 M24 M27	X M2 M7 M11 M24 M27	X M2 M7 M11 M24 M27	X M24 M27

Adapted from CLSI/NCCLS document HS1—*A Quality Management System Model for Health Care.*

Related CLSI Reference Materials*

M2-A9 **Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Ninth Edition (2006).** 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.

M7-A7 **Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Seventh Edition (2006).** This document addresses reference methods for the determination of minimal inhibitory concentrations (MICs) of aerobic bacteria by broth macrodilution, broth microdilution, and agar dilution.

M11-A7 **Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard—Seventh Edition (2007).** This standard provides reference methods for the determination of minimal inhibitory concentrations (MICs) of anaerobic bacteria by agar dilution and broth microdilution.

M23-A2 **Development of *In Vitro* Susceptibility Testing Criteria and Quality Control Parameters; Approved Guideline—Second Edition (2001).** This document addresses the required and recommended data needed for the selection of appropriate interpretive standards and quality control guidelines for new antimicrobial agents.

M24-A **Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes; Approved Standard (2003).** This standard provides protocols and related quality control parameters and interpretive criteria for the susceptibility testing of mycobacteria, *Nocardia* spp., and other aerobic actinomycetes.

M27-A2 **Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard—Second Edition (2002).** 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.

* Proposed-level documents are being advanced through the Clinical and Laboratory Standards Institute consensus process; therefore, readers should refer to the most current editions.

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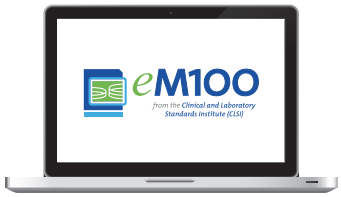

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