CLINICAL AND LABORATORY STANDARDS INSTITUTE®

4th Edition

M27

Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts

This standard covers antifungal agent selection and preparation, test procedure implementation and interpretation, and quality control requirements for susceptibility testing of yeasts that cause invasive fungal infections.

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

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Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts

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Abstract

Clinical and Laboratory Standards Institute standard M27—*Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts* describes a method for testing the susceptibility to antifungal agents of yeasts that cause invasive fungal infections, including *Candida* spp. and *Cryptococcus neoformans*. Selection and preparation of antifungal agents, implementation and interpretation of test procedures, and the purpose and implementation of QC procedures are discussed. A careful examination of the responsibilities of the manufacturer and the user in QC is also presented.

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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. In 1982, the CLSI Area Committee for Microbiology formed the Subcommittee on Antifungal Susceptibility Tests. In 1985, this subcommittee published its first report, in which the results of a questionnaire and a small collaborative study were presented. Based on these findings, the subcommittee concluded that it would be useful to work toward a more reproducible reference testing procedure.

Agreement already existed regarding several elements of the procedure. For example, to facilitate additional analysis of various test conditions, it was agreed that the reference method should be a broth dilution procedure. Because of examples of drug antagonism by some complex media for certain antifungal agents, the subcommittee restricted its interest to fully defined synthetic media only. Drug stock solution preparation and dilution procedures previously developed for antibacterial testing procedures were adopted with minor modifications. Despite agreement in some areas, for other factors, additional data needed to be resolved, including:

- Inoculum preparation
- Inoculum size
- Choice among several synthetic media
- Incubation temperature
- Incubation duration
- End-point definition

These factors were the focus of a series of collaborative studies.[1-4] As a result, the subcommittee reached agreement on all factors, which led to the publication of M27-P in 1992. In the next four years, reference minimal inhibitory concentration (MIC) ranges were established for two QC strains for the available antifungal agents,[5,6] and broth microdilution procedures paralleling the broth macrodilution reference procedure became available.[4,7-9] This information was included in a revised standard in 1995 (M27-T). In revising the standard, the subcommittee focused its attention on developing relevant breakpoints for available antifungal agents,[10] included in M27-A in 1997. Since then, the subcommittee has developed 24- and 48-hour reference MIC ranges for microdilution testing of both established and newly introduced antifungal agents.[11] The study results are included in this standard and CLSI document M60.[12]

Overview of Changes

This standard replaces the previous edition of the approved standard, M27-A3, 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 supplements (M27-S4 and M44-S3) to reflect CLSI document M60,[12] the new supplement for broth dilution and disk diffusion yeast susceptibility testing
  - Added references to epidemiological cutoff values and CLSI documents M57[13] and M59[14]

- **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"
  - Deleted all uses of the phrase "interpretive criteria"
- **Chapter 3, Antifungal Susceptibility Testing Process:**
  - Added an antifungal susceptibility testing process flow chart
  - Replaced procedural text with step-action tables
  - Added an explanation for deleting breakpoints for itraconazole and flucytosine
  - Changed recommended reading time for broth microdilution to 24 hours only for clinical isolates and QC strains (24 and/or 48 hours was accepted for some antifungal agents in M27-A3)
  - Deleted results interpretation information for ketoconazole

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 macrodilution, broth microdilution, susceptibility testing, yeasts

Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts

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 a reference method for testing susceptibility to antifungal agents of yeasts that cause infections, including *Candida* spp. and *Cryptococcus* spp. The intended users are laboratory personnel who perform antifungal susceptibility testing on yeasts. The focus is on developing relevant breakpoints for available antifungal agents[10] and reference minimal inhibitory concentration (MIC) ranges for broth dilution testing of both established and newly introduced antifungal agents.[11] For MIC breakpoints, interpretive categories, and MIC ranges for QC isolates, refer to CLSI document M60.[12]

This method has not been extensively validated for the yeast forms of dimorphic fungi, such as *Blastomyces dermatitidis* or *Histoplasma capsulatum.* Also, testing filamentous fungi (moulds) introduces several additional standardization problems not covered by this procedure and is not included. For an antifungal broth dilution susceptibility testing reference method for filamentous fungi, refer to CLSI document M38.[15]

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.

1.2 Background

This standard provides a reference method developed through a consensus process to facilitate agreement among laboratories in measuring yeast susceptibility to antifungal agents. An important use of a reference method is to provide a standard basis from which other methods can be developed, which also results in interlaboratory agreement within specified ranges. For example, broth microdilution methods using an indicator dye to facilitate breakpoint determinations have been configured to produce results paralleling those obtained by the broth microdilution reference method. To the extent that any method produces results concordant with this reference method, it would be considered to conform with this standard.

1.3 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 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.[16] 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.[17]

1.4 Terminology

1.4.1 A Note on Terminology

CLSI, as a global leader in standardization, is firmly committed to achieving global harmonization whenever 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 different countries and regions and that legally required use of terms, regional usage, and different consensus timelines are all important considerations in the harmonization process. CLSI recognizes its important role in these efforts, and its consensus process focuses on harmonization of terms to facilitate the global application of standards and guidelines.

NOTE: Current fungal taxonomy is being revised. Many genera have both a teleomorph (sexual state) and an anamorph (asexual state) name. In this standard, the traditional *Candida* spp. and *Cryptococcus* spp. names are used to provide continuity to both past procedures and associated documents such as CLSI document M60.[12]

1.4.2 Definitions

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

breakpoint – minimal inhibitory concentration (MIC) or zone diameter value used to categorize an organism as susceptible, susceptible-dose dependent, intermediate, or resistant; **NOTE 1:** MIC or zone diameter values generated by a susceptibility test can be interpreted based upon established breakpoints; **NOTE 2:** See **interpretive category.**

epidemiological cutoff value (ECV) – the minimal inhibitory concentration (MIC) or zone diameter value that separates microbial populations into those with and without acquired and/or mutational resistance based on their phenotypes (wild-type or non-wild-type). The ECV defines the upper limit of susceptibility for the wild-type population of isolates.

EXAMPLE:

Interpretive Category	ECVs	
	MIC, µg/mL	Zone Diameter, mm
Wild-type	≤4	≥20
Non-wild-type	≥8	≤19

