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

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.

A standard for global application developed through the NCCLS consensus process.

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

Abstract

NCCLS document M27-A2—*Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard—Second Edition* describes a method for testing the susceptibility of antifungal agents to yeast that cause invasive fungal infections, including *Candida* species (and *Candida glabrata)*, and *Cryptococcus neoformans*. Selection and preparation of antifungal agents, 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 aids to guide in the selection of antifungal therapy have gained greater attention. In 1982, the NCCLS Area Committee for Microbiology formed the Subcommittee on Antifungal Susceptibility Testing. In 1985, this subcommittee published its first report[1] in which the results of a questionnaire and a small collaborative study were presented. These results are summarized as follows:

- Approximately 20% of the responding NCCLS membership whose hospitals had greater than 200 beds were performing antifungal testing.
- Most testing involved broth dilution methodology.
- Most strains tested were *Candida albicans* or other species of yeasts.
- Most centers tested only a few isolates per year.
- Agreement in minimal inhibitory concentration (MIC) results among several laboratories that participated in a collaborative study was unacceptably low.

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. To facilitate further analysis of various test conditions, the reference method should be a broth macrodilution procedure. Because of examples of drug antagonism by some complex media for certain antifungals, the subcommittee restricted its interest only to fully defined synthetic media. Drug stock solution preparation and dilution procedures previously developed for antibacterial testing procedures were adopted with minor modifications.

Despite agreement in some areas, other factors required additional data to be resolved. These included inoculum preparation; inoculum size; choice among several synthetic media; temperature of incubation; duration of incubation; and end-point definition. These factors were the focus of a series of collaborative studies.[2,3,4,5] As a result; agreement within the subcommittee was achieved on all of the factors and led to the publication of M27-P in 1992. In the next four years (1992-1996), reference MIC ranges were established for two quality control strains for the available antifungal agents,[6,7] and broth microdilution procedures paralleling the broth macrodilution reference procedure became available.[5,8,9,10] This information was included in a revised standard in 1995 (M27-T). In further revising the document, the subcommittee focused its attention on developing relevant breakpoints for available antifungal agents,[11] included in M27-A (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.[12] The results of these studies are included in the current M27-A2 document.

Standard Precautions

Because it is often impossible to know what might be infectious, all human blood specimens are to be treated as infectious and handled according to “standard precautions.” Standard precautions are new guidelines that combine the major features of “universal precautions and body substance isolation” practices. Standard precautions cover the transmission of any pathogen and thus are more comprehensive than universal precautions which are intended to apply only to transmission of blood-borne pathogens. Standard precaution and universal precaution guidelines are available from the U.S. Centers for Disease Control and Prevention (*Guideline for Isolation Precautions in Hospitals*. Infection Control and Hospital Epidemiology. CDC. 1996;Vol 17;1:53-80), (MMWR 1987;36[suppl 2S]2S-18S), and (MMWR

1988;37:377-382, 387-388). For specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials and for recommendations for the management of blood-borne exposure, refer to the most current edition of NCCLS document M29—*Protection of Laboratory Workers from Occupationally Acquired Infections.*

Key Words

Antifungal, broth macrodilution, broth microdilution, susceptibility testing, yeasts

The Quality System Approach

NCCLS subscribes to a quality 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 through a gap analysis. The approach is based on the model presented in the most current edition of NCCLS HS1—*A Quality System Model for Health Care.* The quality system approach applies a core set of "quality system essentials (QSEs)," basic to any organization, to all operations in any healthcare service's path of workflow. The QSEs provide the framework for delivery of any type of product or service, serving as a manager's guide. The quality system essentials (QSEs) are:

QSEs

Documents & Records	Information Management
Organization	Occurrence Management
Personnel	Assessment
Equipment	Process Improvement
Purchasing & Inventory	Service & Satisfaction
Process Control	Facilities & Safety

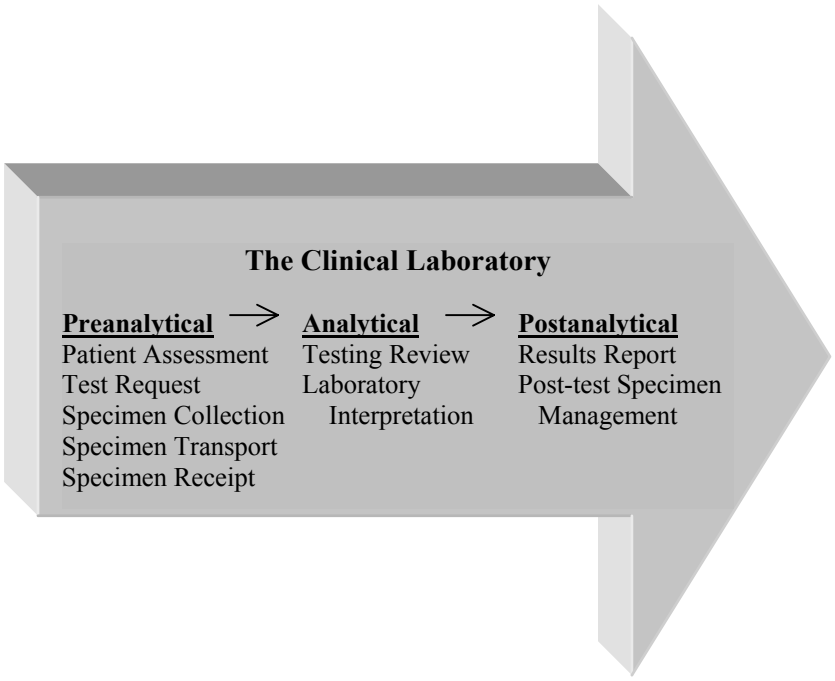
M27-A2 Addresses the Following Quality System Essentials (QSEs):

Documents & Records	Organization	Personnel	Equipment	Purchasing & Inventory	Process Control	Information Management	Occurrence Management	Assessment	Process Improvement	Service & Satisfaction	Facilities & Safety
					X						

Adapted from NCCLS document HS1—*A Quality 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, GP26-A2 defines a clinical laboratory path of workflow which consists of three sequential processes: preanalytical, analytical, and postanalytical. All clinical laboratories follow these processes to deliver the laboratory's services, namely quality laboratory information. The arrow depicts the sequence, from left to right, that any clinical laboratory follows. In addition, the necessary steps or subprocesses are listed below them.



Adapted from NCCLS document HS1-A—*A Quality System Model for Health Care*

Most of NCCLS's documents relate to the clinical laboratory, so the most common path of workflow will be that depicted above. The path of workflow for other healthcare activities, e.g., respiratory services, imaging services, etc., or for other types of organizations, e.g., medical device manufacturers, will differ from that of the clinical laboratory. All such paths of workflow describe the sequence of activities necessary to produce the organization's or an entity's specific product or services. For those documents that relate to other paths of workflow, the icon will reflect different process steps.

M27-A2 Addresses the Following Steps Within the Clinical Laboratory Path of Workflow

Preanalytical					**Analytical**		**Postanalytical**	
Patient Assessment	Test Request	Specimen Collection	Specimen Transport	Specimen Receipt	Testing Review	Laboratory Interpre-tation	Results Report	Post-test Specimen Management
					X	**X**	**X**	**X**

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

1 Introduction

The method described in this document is intended for testing yeasts that cause invasive infections. These yeasts encompass *Candida* species (including *Candida glabrata)* and *Cryptococcus neoformans*. The method has not been used in studies of the yeast form of dimorphic fungi, such as *Blastomyces dermatitidis* or *Histoplasma capsulatum* variety *capsulatum*. Moreover, testing filamentous fungi (moulds) introduces several additional problems in standardization not addressed by the current procedure. A reference method for broth dilution antifungal susceptibility testing of filamentous fungi has been developed and is now available as NCCLS document M38—*Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi*.[13,14]

M27-A2 is a "reference" standard being developed through a consensus process to facilitate the agreement among laboratories in measuring the susceptibility of yeasts 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 will result in interlaboratory agreement within specified ranges. For example, broth microdilution methods, described in this document, have been configured to produce results paralleling those obtained by the reference method. 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 M27-A2.

1.1 Scope

This document describes a method for testing the susceptibility to antifungal agents of yeast that cause infections, including *Candida* species and *Cryptococcus neoformans*. This method has not been extensively validated in yeast form of dimorphic fungi, such as *Blastomyces dermatitidis* or *Histoplasma capsulatum* variety *capsulatum*.

The subcommittee has focused on developing relevant breakpoints for available antifungal agents,[11] and reference MIC ranges for microdilution testing of both established and newly introduced antifungal agents.[12]

1.2 Definitions[a]

Antibiogram, *n* – Overall profile of antimicrobial susceptibility results of a microbial species to a battery of antimicrobial agents.

Minimal inhibitory concentration (MIC), *n* – The lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism in an agar or broth dilution susceptibility test.

[a] Some of these definitions are found in NCCLS document NRSCL8—*Terminology and Definitions for Use in NCCLS Documents*. For complete definitions and detailed source information, please refer to the most current edition of that document.

