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.
NCCLS... Serving the World’s Medical Science Community Through Voluntary Consensus

NCCLS is an international, interdisciplinary, nonprofit, standards-developing, and educational organization that promotes the development and use of voluntary consensus standards and guidelines within the healthcare community. It is recognized worldwide for the application of its unique consensus process in the development of standards and guidelines for patient testing and related healthcare issues. NCCLS is based on the principle that consensus is an effective and cost-effective way to improve patient testing and healthcare services.

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- the authorization of a project
- the development and open review of documents
- the revision of documents in response to comments by users
- the acceptance of a document as a consensus standard or guideline.

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VOLUNTEER PARTICIPATION

Healthcare professionals in all specialties are urged to volunteer for participation in NCCLS projects. Please contact the NCCLS Executive Offices for additional information on committee participation.

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.


THE NCCLS consensus process, which is the mechanism for moving a document through two or more levels of review by the healthcare community, is an ongoing process. Users should expect revised editions of any given document. Because rapid changes in technology may affect the procedures, methods, and protocols in a standard or guideline, users should replace outdated editions with the current editions of NCCLS documents. Current editions are listed in the NCCLS Catalog, which is distributed to member organizations, and to nonmembers on request. If your organization is not a member and would like to become one, and to request a copy of the NCCLS Catalog, contact the NCCLS Executive Offices. Telephone: 610.688.0100; Fax: 610.688.0700; E-Mail: exoffice@nccls.org; Website: www.nccls.org

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Michael A. Pföller, M.D., Chairholder
Vishnu Chaturvedi, Ph.D.
Ana Espinel-Ingroff, Ph.D.
Mahmoud A. Ghannoum, M.Sc., Ph.D.
Linda L. Gosey, M.T.(ASCP)
Frank C. Odds, Ph.D., FRC Path.
John H. Rex, M.D.
Michael G. Rinaldi, Ph.D.
Daniel J. Sheehan, Ph.D.
Thomas J. Walsh, M.D.
David W. Warnock, Ph.D., FRC Path.
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Committee Membership

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James H. Jorgensen, Ph.D.  
Chairholder  
University of Texas Health Center  
San Antonio, Texas

Mary Jane Ferraro, Ph.D., M.P.H.  
Vice-Chairholder  
Massachusetts General Hospital  
Boston, Massachusetts

Subcommittee on Antifungal Susceptibility Tests

Michael A. Pfaller, M.D.  
Chairholder  
University of Iowa College of Medicine  
Iowa City, Iowa

Vishnu Chaturvedi, Ph.D.  
New York State Department of Health  
Albany, New York

Ana Espinel-Ingroff, M.S., Ph.D.  
Medical College of Virginia/VCU  
Richmond, Virginia

Mahmoud A. Ghannoum, M.Sc., Ph.D.  
Center for Medical Mycology, Case Western Reserve University, and University Hospitals of Cleveland  
Cleveland, Ohio

Linda L. Gosey, M.T.(ASCP)  
Food and Drug Administration  
Rockville, Maryland

Frank C. Odds, Ph.D., FRC Path.  
University of Aberdeen  
Scotland, United Kingdom

John H. Rex, M.D.  
University of Texas Health Science Ctr. at Houston  
Houston, Texas

Michael G. Rinaldi, Ph.D.  
University of Texas Health Science Center  
San Antonio, Texas

Daniel J. Sheehan, Ph.D.  
Pfizer Inc.  
New York, New York

Thomas J. Walsh, M.D.  
National Cancer Institute  
Bethesda, Maryland

David W. Warnock, Ph.D., FRC Path.  
Centers for Disease Control and Prevention  
Atlanta, Georgia

Advisor

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Clinical Microbiology Institute  
Wilsonville, Oregon
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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 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. 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, and broth microdilution procedures paralleling the broth macrodilution reference procedure became available. 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, 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. 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-1S), and (MMWR
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 fromOccupationally 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:

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

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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**

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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

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.

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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.