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

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 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 Filamentous Fungi; Approved Standard

Abstract

NCCLS document M38-A—*Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard* describes a method for testing the susceptibility of filamentous fungi (moulds) that cause invasive fungal infections, including *Aspergillus* species, *Fusarium* species, *Rhizopus arrhizus, Pseudallescheria boydii (Scedosporium apiospermum),* and *Sporothrix schenckii* and other opportunistic pathogenic moulds 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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Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard

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Foreword

With the increased incidence of systemic fungal infections and the growing number of antifungal agents, laboratory methods to guide in the selection of antifungal therapy have gained greater attention. The NCCLS Area Committee on Microbiology formed the Subcommittee on Antifungal Susceptibility Testing and data were collected for testing yeasts in a series of collaborative studies. As a result, NCCLS document M27—*Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeast,* was published with the establishment of 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 two collaborative studies, agreement within the subcommittee was achieved regarding testing conditions that included inoculum preparation and inoculum size, incubation time and temperature, medium formulation, and criteria for MIC determination.[1,2] An additional study has indicated some degree of correlation between *in vitro* test results and response to treatment in animal models. [2,3]

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.[1,2] The subcommittee has evaluated other medium formulations, but the standard RPMI medium facilitated a more consistent identification of itraconazole resistance in *Aspergillus* spp. in eight laboratories.[4] Drug stock solution preparation and dilution procedures previously developed for antifungal testing of yeasts procedures (M27) also were adopted.

Standard Precautions

Because it is often impossible to know what might be infectious, all human blood, fluid, or tissue 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 NCCLS document M29—*Protection of Laboratory Workers from Occupationally Acquired Infections*.

Key Words

Antifungal, broth microdilution, filamentous fungi or moulds, susceptibility testing

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 document 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

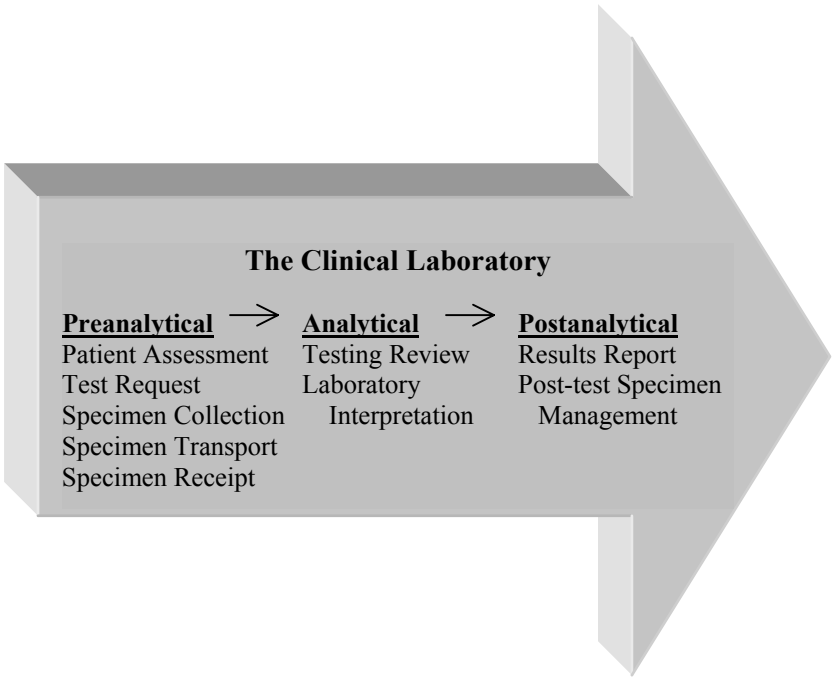
M38-A Addresses the following Quality System Essentials (QSEs)

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—*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 an 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.

If the document is specific to clinical laboratory processes or procedures, the following chart will indicate which process step(s) are included within the specific document.

M38-A 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 Filamentous Fungi; Approved Standard

1 Introduction

The method described in this document is intended for testing the more common filamentous fungi or moulds that cause invasive infections. These moulds encompass *Aspergillus* species, *Fusarium* species, *Rhizopus* species, *Pseudallescheria boydii*, and the mycelial form of *Sporothrix schenckii*. Although other opportunistic monilaceous and dematiaceous moulds have been evaluated,[5] caution should be used when interpreting the MIC results from other mould/drug combinations. The method has not been used in studies of the yeast form of dimorphic fungi, such as *Blastomyces dermatitidis*, or *Coccidioides immitis*, *Histoplasma capsulatum* variety *capsulatum*, or *Penicillium marneffei*, or *S. schenckii*.

NCCLS document M38-A 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 to be emphasized that the relationship between *in vitro* versus *in vivo* data has only been attempted in animal models.[3] 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 NCCLS document M38-A.

1.1 Scope

This document describes a method for testing the susceptibility of filamentous fungi (moulds) that cause invasive fungal infections, including *Aspergillus* species, *Fusarium* species, *Rhizopus arrhizus, Pseudallescheria boydii,* and *Sporothrix schenckii* and other pathogenic moulds to antifungal agents. Addressed in this document are testing conditions including inoculum preparation and inoculum size, incubation time and temperature, medium formulation, and criteria for MIC determination.

This standard focuses on the fully defined synthetic medium RPMI-1640 for testing of moulds because of examples of the suitability of this test medium for antifungal susceptibility testing of yeast.[1,2]

NCCLS document M27—*Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeast,* is referenced for drug stock solution preparation and dilution procedures.

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.