M44-A
Vol. 24 No. 15
Replaces M44-P
Vol. 23 No. 6

Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guideline

This document provides newly established methodology for disk diffusion testing of *Candida* spp., criteria for quality control testing, and interpretive criteria.

A guideline 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.

In addition to developing and promoting the use of voluntary consensus standards and guidelines, NCCLS provides an open and unbiased forum to address critical issues affecting the quality of patient testing and health care.

PUBLICATIONS

An NCCLS document is published as a standard, guideline, or committee report.

Standard A document developed through the consensus process that clearly identifies specific, essential requirements for materials, methods, or practices for use in an unmodified form. A standard may, in addition, contain discretionary elements, which are clearly identified.

Guideline A document developed through the consensus process describing criteria for a general operating practice, procedure, or material for voluntary use. A guideline may be used as written or modified by the user to fit specific needs.

Report A document that has not been subjected to consensus review and is released by the Board of Directors.

CONSENSUS PROCESS

The NCCLS voluntary consensus process is a protocol establishing formal criteria for:

- 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.

Most NCCLS documents are subject to two levels of consensus—"proposed" and "approved." Depending on the need for field evaluation or data collection, documents may also be made available for review at an intermediate (i.e., "tentative") consensus level.

Proposed An NCCLS consensus document undergoes the first stage of review by the healthcare community as a proposed standard or guideline. The document should receive a wide and thorough technical review, including an overall review of its scope, approach, and utility, and a line-by-line review of its technical and editorial content.

Tentative A tentative standard or guideline is made available for review and comment only when a recommended method has a well-defined need for a field evaluation or when a recommended protocol requires that specific data be collected. It should be reviewed to ensure its utility.

Approved An approved standard or guideline has achieved consensus within the healthcare community. It should be reviewed to assess the utility of the final document, to ensure attainment of consensus (i.e., that comments on earlier versions have been satisfactorily addressed), and to identify the need for additional consensus documents.

NCCLS standards and guidelines represent a consensus opinion on good practices and reflect the substantial agreement by materially affected, competent, and interested parties obtained by following NCCLS's established consensus procedures. Provisions in NCCLS standards and guidelines may be more or less stringent than applicable regulations. Consequently, conformance to this voluntary consensus document does not relieve the user of responsibility for compliance with applicable regulations.

COMMENTS

The comments of users are essential to the consensus process. Anyone may submit a comment, and all comments are addressed, according to the consensus process, by the NCCLS committee that wrote the document. All comments, including those that result in a change to the document when published at the next consensus level and those that do not result in a change, are responded to by the committee in an appendix to the document. Readers are strongly encouraged to comment in any form and at any time on any NCCLS document. Address comments to the NCCLS Executive Offices, 940 West Valley Road, Suite 1400, Wayne, PA 19087, USA.

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.

M44-A
ISBN 1-56238-532-1
Volume 24 Number 15 ISSN 0273-3099

Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guideline

Daniel J. Sheehan, Ph.D.
Steven D. Brown, Ph.D.
Michael A. Pfaller, M.D.
David W. Warnock, Ph.D., FRCPath.
John H. Rex, M.D., FACP
Vishnu Chaturvedi, Ph.D.
Ana Espinel-Ingroff, Ph.D.
Mahmoud A. Ghannoum, M.Sc., Ph.D.
Lynn Steele Moore, M.T.
Frank C. Odds, Ph.D., FRCPath.
Michael G. Rinaldi, Ph.D.
Thomas J. Walsh, M.D.

Abstract

NCCLS broth dilution reference methods are available for susceptibility testing of yeasts (see NCCLS document M27—*Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts*) and moulds (see NCCLS document M38—*Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi*). There still remains, however, a need for an alternative simple, rapid, and cost-effective approach to determine susceptibility of fungal organisms to various classes of antifungal agents that would make antifungal susceptibility testing more readily available to the clinical microbiology laboratory. The NCCLS Subcommittee on Antifungal Susceptibility Testing has developed a disk diffusion method for testing *Candida* species to fluconazole and voriconazole. Zone interpretive criteria (breakpoints) have been approved for fluconazole as well as quality control parameters for both fluconazole and voriconazole. One significant advantage of this method is that qualitative results can usually be determined after only 20 to 24 hours incubation as opposed to 48 hours with NCCLS document M27. There are currently more than ten systemically active antifungal agents and it is expected that this document will further encourage the development of disk diffusion testing for at least some of these agents.

NCCLS. *Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guideline*. NCCLS document M44-A (ISBN 1-56238-532-1). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2004.

Suggested Citation

(NCCLS. *Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guideline.* NCCLS document M44-A [ISBN 1-56238-532-1]. NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2004.)

Proposed Guideline
April 2003

Approved Guideline
May 2004

ISBN 1-56238-532-1
ISSN 0273-3099

Committee Membership

Area Committee on Microbiology

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

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

Donald R. Callihan, Ph.D.
BD Diagnostic Systems
Sparks, Maryland

David L. Sewell, Ph.D.
Veterans Affairs Medical Center
Portland, Oregon

Thomas R. Shryock, Ph.D.
Lilly Research Laboratories

Jana M. Swenson, M.M.Sc.
Centers for Disease Control and Prevention
Atlanta, Georgia

Michael L. Wilson, M.D.
Denver Health Medical Center
Denver, Colorado

Advisors

Ellen Jo Baron, Ph.D.
Stanford University Hospital & Medical School
Stanford, California

Lynne S. Garcia, M.S.
LSG & Associates
Santa Monica, California

Richard L. Hodinka, Ph.D.
Children's Hospital of Philadelphia
Philadelphia, Pennsylvania

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

Robert P. Rennie, Ph.D.
Provincial Laboratory for Public Health
Edmonton, AB, Canada

Melvin P. Weinstein, M.D.
Robert Wood Johnson Medical School
New Brunswick, New Jersey

Gail L. Woods, M.D.
ARUP Research Institute
Salt Lake City, Utah

Subcommittee on Antifungal Susceptibility Tests

John H. Rex, M.D., FACP
Chairholder
AstraZeneca
Cheshire, United Kingdom

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

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

Mahmoud A. Ghannoum, M.Sc., Ph.D.
Case Western Reserve University
Cleveland, Ohio

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

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

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

Lynn Steele-Moore, M.T.
FDA Center for Drug Evaluation & Research
Rockville, Maryland

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

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

Advisor

Arthur L. Barry, Ph.D.
The Clinical Microbiology Institute
Wilsonville, Oregon

Working Group on Antifungal Disk Diffusion Susceptibility Testing of Yeasts

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

Steven D. Brown, Ph.D.
The Clinical Microbiology Institute
Wilsonville, Oregon

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

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

Staff

Tracy A. Dooley, M.L.T.(ASCP)
Staff Liaison
NCCLS
Wayne, Pennsylvania

Donna M. Wilhelm
Editor
NCCLS
Wayne, Pennsylvania

Melissa A. Lewis
Assistant Editor
NCCLS
Wayne, Pennsylvania

Contents

Contents (Continued)

Foreword

Due to the increased incidence of systemic fungal infections and number of antifungal agents available for systemic administration, antifungal susceptibility testing has gained greater recognition. Today, antifungal susceptibility testing has come of age in guiding physicians in the selection of antifungal therapy. Broth macrodilution and microdilution reference methods are now available for susceptibility testing of both yeasts (see NCCLS document M27—*Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts*) and moulds (see NCCLS document M38—*Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi*). A commercial broth microdilution panel has been cleared for clinical use by the FDA. To make antifungal susceptibility testing more readily available to clinical microbiology laboratories, there still remains a need for alternative, simple, rapid, and cost-effective approaches. Disk diffusion testing has served as such an example for antibacterial testing (see NCCLS document M2—*Performance Standards for Antimicrobial Disk Susceptibility Tests*) and, therefore, the NCCLS Subcommittee on Antifungal Susceptibility Tests has developed recommendations for disk diffusion testing for antifungal agents.

A disk diffusion method for testing *Candida* species against the triazoles (fluconazole and voriconazole) has been developed. This method often provides qualitative results 24 hours sooner than the standard method for testing yeasts used in NCCLS document M27—*Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts.* In addition, the use of supplemented Mueller-Hinton agar in lieu of RPMI 1640 medium should make antifungal susceptibility testing more readily available to at least some clinical laboratories, and at reduced cost. Zone interpretive criteria (breakpoints) for fluconazole and quality control parameters for both fluconazole and voriconazole have been established according to standard NCCLS procedures. NCCLS expects that this document will encourage the development of disk diffusion testing for other antifungal agents and fungal genera.

A Note on Terminology

NCCLS, as a global leader in standardization, is firmly committed to achieving global harmonization wherever possible. Harmonization is a process of recognizing, understanding, and explaining differences while taking steps to achieve worldwide uniformity. NCCLS recognizes that medical conventions in the global metrological community have evolved differently in the United States, Europe, and elsewhere; that these differences are reflected in NCCLS, International Organization for Standardization (ISO), and European Committee for Standardization (CEN) documents; and that legally required use of terms, regional usage, and different consensus timelines are all obstacles to harmonization. In light of this, NCCLS recognizes that harmonization of terms facilitates the global application of standards and is an area of immediate attention. Implementation of this policy must be an evolutionary and educational process that begins with new projects and revisions of existing documents.

Of particular note in NCCLS document M44-A are two terms whereby NCCLS intends to eliminate confusion, over time, through its commitment to harmonization. For the most part, in this guideline, the term “accuracy” is used correctly in its metrological sense, to refer to the closeness of the agreement between the result of a (single) measurement and a true value of a measurand, thus comprising both random and systematic effects. But there are several instances in this document where accuracy is defined the way ISO defines “trueness,” i.e., the closeness of the agreement between the average value from a large series of measurements and true value of a measurand. To facilitate understanding, when used this way, “trueness” has been inserted parenthetically. Also, the terms are defined in the guideline’s Definitions section along with explanatory notes. During the next scheduled revision of this document, they will be reviewed for consistency with international use, and revised appropriately.

Key Words

Antifungal, antimicrobial, disk, disk diffusion, Kirby-Bauer method, susceptibility testing

Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guideline

1 Scope

With a need to make antifungal susceptibility testing more readily available to the clinical laboratory, NCCLS document M44—*Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts* provides an established methodology for disk diffusion testing of *Candida* spp., zone interpretive criteria for fluconazole, and recommended quality control ranges for fluconazole and voriconazole.

2 Introduction

The method described in this document is intended for testing *Candida* species. This method does not currently encompass any other genera and has not been used in studies of the yeast form of dimorphic fungi, such as *Blastomyces dermatitidis* or *Histoplasma capsulatum.* Moreover, testing of filamentous fungi (i.e., moulds) is not addressed in the current procedure.

The method described herein must be followed exactly to obtain reproducible results. When new problems are recognized or improvements in these criteria are developed, changes will be incorporated into future editions of this guideline and also distributed in periodic informational supplements.

3 Standard Precautions

Because it is often impossible to know what might be infectious, all human specimens are to be 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 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.*

4 Definitions

Antimicrobial Susceptibility Test Interpretive Category – 1) A classification based on an *in vitro* response of an organism to an antimicrobial agent at levels of that agent corresponding to blood or tissue levels attainable with usually prescribed doses of that agent; **2) Susceptible Antimicrobial Susceptibility Test Interpretive Category** – A category that implies that an infection due to the isolate may be appropriately treated with the dosage of an antimicrobial agent recommended for that type of infection and infecting species, unless otherwise contraindicated; **3) Susceptible-Dose Dependent (S-DD) Antimicrobial Susceptibility Test Interpretive Category** – A category that includes isolates with antimicrobial agent MICs that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates; **4) Resistant Antimicrobial Susceptibility Test Interpretive Category** – Resistant isolates that are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules or where clinical efficacy has not been reliable in treatment studies.

Quality control – Part of quality management focused on fulfilling quality requirements (ISO 9000:2000 [3.1.2])[1]; **NOTE:** This includes operational techniques and activities used to fulfill these requirements.

Trueness – The closeness of agreement between the average value obtained from a large series of test results and an accepted reference value (ISO 3534-1 [3.12]).[2]

5 Selection of Antimicrobial Agent Disks for Routine Testing and Reporting

Although interpretive criteria are now available for a limited number of organism-drug combinations, routine testing is not generally recommended. However, some institutions may find it useful to systematically test selected drug-organism combinations (e.g., fluconazole vs. *Candida* from sterile sites). At each institution, the decision to perform testing of yeast isolates is best made as a collaborative effort among infectious disease practitioners, the pharmacy and therapeutics committee, clinical microbiology personnel, and the infection control committee.

5.1 Use of Nonproprietary or Generic Names

To minimize confusion, all antifungal agents should be referred to by international nonproprietary (i.e., generic) names.

5.2 Number of Agents Tested

To make routine susceptibility tests relevant and practical, the number of antimicrobial agents tested should be limited. Although this is not an immediate issue for antifungal agents, the same principle would apply.

5.3 Suggested Guidelines for Selective Reporting

Testing may be warranted under certain selected circumstances such as: (a) part of periodic batch surveys that establish antibiograms for collections of pathogenic isolates obtained from within an institution; (b) to aid in the management of refractory oropharyngeal infections due to *Candida* spp. in patients experiencing therapeutic failure with the standard agent at the standard dose; and (c) to aid in the management of invasive infections due to *Candida* spp. when the utility of azole antifungal agents is uncertain (e.g., when the infection is due to a species other than *C. albicans*). Disk diffusion interpretive criteria are available only for fluconazole vs. *Candida* spp., and the clinical relevance of testing any other drug-organism combination remains uncertain.

6 Equipment/Materials

The following equipment is recommended for performance of antifungal disk diffusion susceptibility testing:

- Incubator set at 35 ^{o}C ($\pm$2 ^{o}C) with ambient air;
- McFarland 0.5 Turbidity Standard;
- Sterile cotton (not synthetic polyester fiber) swabs; and
- Sterile physiologic (8.5 g/L NaCl; 0.85%) saline.

7 Reagents for the Disk Diffusion Test

7.1 Mueller-Hinton Agar + 2% Glucose and 0.5 µg/mL Methylene Blue Dye (GMB) Medium (See Appendix A)

Of the many agar media available, the subcommittee considers supplemented Mueller-Hinton agar to be a good choice for routine susceptibility testing of yeasts for the following reasons:

- It is readily available.
- It shows acceptable batch-to-batch reproducibility.[3,4]
- When supplemented with glucose to a final concentration of 2%, it provides for suitable fungal growth. The addition of methylene blue dye to a final concentration of 0.5 µg/mL enhances zone edge definition.
- The base medium can easily be supplemented either pre- or postproduction to contain the final concentration of 2% glucose and 0.5 µg/mL methylene blue dye.

Although Mueller-Hinton agar is generally reliable for susceptibility testing, results obtained with some brands and batches may, on occasion, vary significantly. If a batch of medium does not support adequate growth of a test organism, zones obtained in a disk diffusion test will usually be larger than expected and may exceed the acceptable quality control limits. Only Mueller-Hinton medium formulations that have been tested and meet the acceptance limits described in NCCLS document M6—*Protocols for Evaluating Dehydrated Mueller-Hinton Agar* should be used.

7.1.1 pH of Mueller-Hinton Agar + 2% Glucose and 0.5 µg/mL Methylene Blue Dye Medium

The pH of each batch of prepared Mueller-Hinton agar should be checked. The method used will largely depend on the type of equipment available in the laboratory. The agar medium should have a pH between 7.2 and 7.4 at room temperature after gelling. The pH can be checked by one of the following means:

- Macerate a sufficient amount of agar to submerge the tip of a pH electrode.
- Allow a small amount of agar to solidify around the tip of a pH electrode in a beaker or cup.
- Use a properly calibrated surface electrode.

7.1.2 Moisture on Agar Surface

If excess surface moisture is present, the agar plates should be dried in an incubator or laminar flow hood with the lids ajar until the excess moisture has evaporated (usually 10 to 30 minutes). The surface should be moist, but with no droplets on the agar surface or the petri dish cover.

7.1.3 Storage of Antimicrobial Disks

Cartridges containing commercially prepared paper disks specifically for susceptibility testing are generally packaged to ensure appropriate anhydrous conditions. Disks should be stored as follows:

- Refrigerate the containers at 8 °C or below, or freeze at -14 °C or below, in a nonfrost-free freezer until needed. The disks may retain greater stability if stored frozen until the day of use. Always refer to instructions in the product insert.

- The unopened disk containers should be removed from the refrigerator or freezer one to two hours before use so they may equilibrate to room temperature before opening. This procedure minimizes the amount of condensation that occurs when warm air contacts cold disks.

- Once a cartridge of disks has been removed from its sealed packaging, it should be placed in a tightly sealed, desiccated container.

- A disk-dispensing apparatus should be fitted with a tight cover and supplied with an adequate desiccant. The dispenser should be allowed to warm to room temperature before opening. The desiccant should be replaced when the indicator changes color.

- When not in use, the dispensing apparatus containing the disks should always be refrigerated.

- Only disks within their valid shelf life may be used. Disks should be discarded on the expiration date.

7.2 Turbidity Standard for Inoculum Preparation

To standardize the inoculum density for a susceptibility test, a $BaSO_4$ suspension with a turbidity, equivalent to a 0.5 McFarland standard or its optical equivalent (e.g., latex particle suspension), should be used. See Appendix B for instructions on preparing a $BaSO_4$ turbidity standard.

8 Procedure for Performing the Disk Diffusion Test

8.1 Inoculum Preparation: Direct Colony Suspension Method

Steps for preparation of the inoculum are as follows:

(1) All organisms need to be subcultured onto blood agar or Sabouraud dextrose agar to ensure purity and viability. The incubation temperature throughout must be 35 °C (±2 °C).

(2) Inoculum is prepared by picking five distinct colonies of approximately 1 mm in diameter from a 24-hour-old culture of *Candida* species. Colonies are suspended in 5 mL of sterile 0.145 mol/L saline (8.5 g/L NaCl; 0.85% saline).

(3) The resulting suspension is vortexed for 15 seconds and its turbidity is adjusted either visually or with a spectrophotometer by adding sufficient sterile saline or more colonies to adjust the transmittance to that produced by a 0.5 McFarland standard (see Appendix B) at 530 nm wavelength. This procedure will yield a yeast stock suspension of 1×10^6 to 5×10^6 cells per mL and should produce semi-confluent growth with most *Candida* species isolates.

8.2 Inoculation of Test Plates

(1) Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab is dipped into the suspension. The swab should be rotated several times and pressed firmly against the inside wall of the tube above the fluid level. This will remove excess fluid from the swab.

(2) The dried surface of a sterile Mueller-Hinton + GMB agar plate is inoculated by evenly streaking the swab over the entire agar surface. This procedure is repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar is swabbed.

(3) The lid may be left ajar for three to five minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug-impregnated disks.

NOTE: Variations in inoculum density must be avoided. Never use undiluted overnight broth cultures or other unstandardized inocula for streaking plates.

8.3 Application of Disks to Inoculated Agar Plates

(1) Antimicrobial disks are dispensed onto the surface of the inoculated agar plate. Each disk must be pressed down to ensure its complete contact with the agar surface. Whether the disks are placed individually or with a dispensing apparatus, they must be distributed evenly so that they are no closer than 24 mm from center to center. Ordinarily, no more than 12 disks should be placed on a 150-mm plate, or more than five disks on a 100-mm plate. Because the drug diffuses almost instantaneously, a disk should not be moved once it has come into contact with the agar surface. Instead, place a new disk in another location on the agar.

(2) The plates are inverted and placed in an incubator set to 35 °C (± 2 °C) within 15 minutes after the disks are applied.

8.4 Reading Plates and Interpreting Results

Examine each plate after 20 to 24 hours of incubation. If the plate was satisfactorily streaked and the inoculum was correct, the resulting zones of inhibition will be uniformly circular and there will be a semi-confluent lawn of growth. The plate is held a few inches above a black, nonreflecting background illuminated with reflected light. Measure the zone diameter to the nearest whole millimeter at the point at which there is a prominent reduction in growth. This is highly subjective, and experience results in greater accuracy (trueness). Pinpoint microcolonies at the zone edge or large colonies within a zone are encountered frequently and should be ignored. If these colonies are subcultured and retested, identical results are usually obtained, i.e., a clear zone with microcolonies at the zone edge or large colonies within the zone.[5] Read at 48 hours only when insufficient growth is observed after 24 hours incubation.

9 Interpretation of Disk Diffusion Test Results

9.1 Zone Diameter Interpretive Standards

Table 1 provides zone diameter interpretive criteria to categorize accurately the levels of susceptibility of organisms to fluconazole. These categories were developed by first comparing zone diameters to MICs for a large number of isolates, including those with known mechanisms of resistance relevant to the particular drug. MICs and correlated zone sizes were analyzed in relation to the pharmacokinetics of the drug from normal dosing regimens. Whenever possible, the tentative *in vitro* interpretive criteria were analyzed in relation to studies of clinical efficacy in the treatment of specific pathogens.[6-8] (See also NCCLS documents M23—*Development of* In Vitro *Susceptibility Testing Criteria and Quality Control Parameters* and M27—*Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts.)*

Of note, these interpretive breakpoints are not applicable to *C. krusei*, and thus identification to the species level is required in addition to MIC determination.

9.2 Interpretive Categories

9.2.1 Susceptible (S)

The susceptible category implies that an infection due to the strain may be appropriately treated with the dose of antimicrobial agent recommended for that type of infection and infecting species, unless otherwise contraindicated.

9.2.2 Susceptible-Dose Dependent (S-DD)

The susceptible-dose dependent category includes isolates with antimicrobial agent MICs that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates. Susceptibility is dependent on achieving the maximal possible blood level. This category also includes a buffer zone, which should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations, especially for drugs with narrow pharmacotoxicity margins.

9.2.3 Resistant (R)

Resistant strains are those that are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules or when zone diameters have been in a range where clinical efficacy has not been reliable in treatment studies.

9.3 Zone Diameter Interpretive Criteria

Disk diffusion zone diameters correlate inversely with MICs from standard dilution tests. Table 1 lists the zone diameter interpretive criteria. These criteria were based on zone diameter versus MIC comparisons for the MIC interpretive criteria defined in NCCLS document M27—*Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts.*

10 Quality Control Procedures

10.1 Purpose

The goals of a quality control program are to monitor the following:

- the precision (repeatability) and accuracy (trueness) of the susceptibility test procedure;
- the performance of reagents used in the test; and
- the performance of persons who carry out the tests and read results.

These goals are best achieved by, but not limited to, the testing of quality control strains with known susceptibility to the antimicrobial agents being tested.

10.2 Reference Strains for Quality Control

To control the precision (repeatability) and accuracy (trueness) of the results obtained with disk diffusion test procedure, several quality control strains should be obtained from a reliable source. The recommended quality control strains include:

- *Candida albicans* ATCC 90028;

- *Candida parapsilosis* ATCC 22019;
- *Candida tropicalis* ATCC 750; and
- *Candida krusei* ATCC 6258.

10.3 Storing Quality Control Strains

- The quality control strains should be tested by the standard disk diffusion test procedure described herein using the same materials and methods that are used to test clinical isolates.
- Quality control strains are stored in a way that minimizes the possibility of mutation in the organism.
- There are several methods for prolonged storage of reference strains. For example, yeasts may be grown on slants of potato dextrose agar and then frozen at -70 °C as described by Pasarell and McGinnis.[9] Alternatively, strains can be preserved by suspending yeasts into vials containing 50% glycerol solution for freezing and storing at -70 °C. Commercial storage systems are also available.[10]
- Working quality control cultures are stored on blood agar or Sabouraud dextrose agar at 2 to 8 °C and subcultured each week for no more than three successive weeks. New working cultures should be prepared at least monthly from frozen, freeze-dried, or commercial cultures.
- Frozen or freeze-dried cultures should be subcultured at least twice prior to testing.
- A quality control strain can be used to monitor the precision (repeatability) and accuracy (trueness) of the disk test as long as there is no significant change in the mean zone diameter that cannot be attributed to a faulty methodology. If an unexplained result suggests a change in the organism's inherent susceptibility, a fresh new stock culture of the control strain should be obtained.

10.4 Zone Diameter Quality Control Limits

Acceptable zone diameter quality control limits for quality control strains are listed in Table 2. The overall performance of the test system should be monitored using these ranges by testing the appropriate control strains each day the test is performed or, if satisfactory performance is documented (see Section 10.5.2.1), testing may be done weekly (see Section 10.5.2).

10.5 Frequency of Quality Control Testing

10.5.1 Daily Testing

When testing is performed daily, for each antimicrobial agent/organism combination, 1 out of every 20 consecutive results may be out of the acceptable range (based on 95% confidence limits, 1 out of 20 random results may be out of control). Any more than 1 out-of-control result in 20 consecutive tests requires corrective action (see Section 10.6).

10.5.2 Weekly Testing

10.5.2.1 Demonstrating Satisfactory Performance for Conversion from Daily to Weekly Quality Control Testing

- Test all applicable control strains for 20 consecutive test days and document results.

- To convert from daily to weekly quality control testing, no more than 1 out of 20 zone diameters for each antimicrobial agent/organism combination may be outside the acceptable zone diameter limits in Table 2.

10.5.3 Implementing Weekly Quality Control Testing

- Weekly quality control testing may be implemented once satisfactory performance has been documented (see Section 10.5.2.1).

- Perform quality control testing once per week and whenever any reagent component of the test (e.g., a new lot of agar plates or a new lot of disks from the same or a different manufacturer) is changed.

- If any of the weekly quality control results are out of the acceptable range, corrective action is required (see Section 10.6).

- If a new antimicrobial agent is added, it must be tested for 20 consecutive test days and satisfactory performance documented before converting to a weekly schedule. In addition, 20 days of consecutive testing are required if there is a major change in the method of reading test results, such as conversion from manual zone measurements to an automated zone reader.

10.6 Corrective Action

10.6.1 Out-of-Control Result Due to an Obvious Error

Obvious reasons for out-of-control results include:

- use of the wrong disk;

- use of the wrong control strain;

- obvious contamination of the strain; or

- inadvertent use of the wrong incubation temperature or conditions.

In such cases, document the reason and retest the strain on the day the error is observed. If the repeated result is within range, no further corrective action is required.

10.6.2 Out-of-Control Result Not Due to an Obvious Error

10.6.2.1 Immediate Corrective Action

If there is no obvious reason for an out-of-control result, immediate corrective action is required.

- Test the antimicrobial agent/organism combination for a total of five consecutive test days. Document all results in question.

- If all five zone diameter measurements for the antimicrobial agent/organism combination are within acceptable ranges, as defined in Table 2, no additional corrective action is necessary.

- If any of the five zone diameter measurements are outside the acceptable range, additional corrective action is required (see Section 10.6.2.2).

- Daily control tests must be continued until final resolution of the problem can be achieved.

10.6.2.2 Additional Corrective Action

When immediate corrective action does not resolve the problem, it is likely due to a system error versus a random error. The following common sources of error should be investigated:

- Zone diameters were measured and transcribed correctly.
- The turbidity standard has not expired, is stored properly, meets performance requirements (see Section 7.2 and Appendix B), and was adequately mixed prior to use.
- All materials used were within their expiration date and stored at the proper temperature.
- The incubator is at the proper temperature and atmosphere.
- Other equipment used (e.g., pipettors) are functioning properly.
- Disks are stored desiccated and at the proper temperature.
- The control strain has not changed and is not contaminated.
- Inoculum suspensions were prepared and adjusted correctly.
- Inoculum for the test was prepared from a plate incubated for the correct length of time and in no case was more than 24 hours old.

It may be necessary to obtain a new quality control strain (either from freezer stock or a reliable source) and new lots of materials (including new turbidity standards), possibly from different manufacturers. If the problem appears to be related to a commercial product, the manufacturer should be contacted. It is also helpful to exchange quality control strains and test materials with another laboratory using the same method. Until the problem is resolved, an alternative test method should be used.

Once the problem is corrected, documentation of satisfactory performance for another 20 consecutive days is required before returning to weekly quality control testing (see Section 10.5.2.1).

10.7 Reporting Patient Results When Out-of-Control Tests Occur

Whenever an out-of-control result or corrective action is necessary, careful assessment of whether to report patient results should be made on an individual basis, taking into account if the source of error, when known, is likely to have affected relevant patient results. Options that may be considered include suppressing the results for an individual antimicrobial agent; retrospectively reviewing individual patient or cumulative data for unusual patterns; and using an alternate test method or a reference laboratory until the problem is resolved.

11 Limitations of Disk Diffusion Methods

11.1 Application to Various Organism Groups

The disk diffusion method described in this document has been standardized for *Candida* species only. For other yeasts, consultation with an infectious disease specialist is recommended for guidance in determining the need for susceptibility testing and interpretation of results. Published reports in the medical literature and current consensus recommendations for therapy of uncommon microorganisms

may obviate the need for testing. If necessary, a reference dilution method may be the most appropriate alternative testing method, and this may require submitting the organism to a reference laboratory.

11.2 Verification of Patient Results

Multiple test parameters are monitored by following the quality control recommendations described in this standard. However, acceptable results derived from testing quality control strains do not guarantee accurate results when testing patient isolates. It is important to review all of the results obtained from all drugs tested on a patient's isolates prior to reporting the results.

Unusual or inconsistent results should be verified by checking for the following: 1) transcription errors; 2) contamination of the test (recheck purity plates); and 3) previous results on the patient's isolates. If a reason for the unusual or inconsistent result cannot be ascertained, repeat the susceptibility test, verify the species identity, or request a new clinical specimen. Each laboratory must develop its own policies for verification of unusual or inconsistent antimicrobial susceptibility test results.

Table 1. Zone Diameter Interpretive Standards and Corresponding Minimal Inhibitory Concentrations (MIC) Breakpoints for *Candida* spp.

Antifungal Agent	Disk Content	Zone Diameter, Nearest Whole (mm)			Equivalent MIC Breakpoints (µg/mL)		
		R*	S-DD*	S*	R*	S-DD*	S*
Fluconazole†	25 µg	≤14	15 - 18	≥19	≥64	16 - 32	≤8

* Susceptible, Susceptible-Dose Dependent (S-DD), and Resistant Interpretive categories are defined in Section 9.2.

† Isolates of *C. krusei* are assumed to be intrinsically resistant to fluconazole, and their MICs should not be interpreted using this scale.

Table 2. Recommended Quality Control Zone Diameter (mm) Ranges

Antifungal Agent	Disk Content	*C. albicans* ATCC 90028	*C. parapsilosis* ATCC 22019	*C. tropicalis* ATCC 750	*C. krusei* ATCC 6258
Fluconazole	25 µg	28 - 39	22 - 33	26 - 37	—*
Voriconazole	1 µg	31 - 42	28 - 37	—*	16 - 25

*Quality control ranges have not been established for these strain/antimicrobial agent combinations, due to their extensive interlaboratory variation during initial quality control studies.

References

[1] ISO 9000. *Quality Management Systems Fundamentals and Vocabulary*. Geneva: International Organization for Standardization; 2000.

[2] ISO 3534-1. *Statistics—Vocabulary and symbols.* Geneva: International Organization for Standardization; 1993.

[3] Barry AL, Pfaller MA, Rennie RP, Fuchs PC, Brown SD. Precision and accuracy of fluconazole susceptibility testing by broth microdilution, Etest, and disk diffusion methods. *Antimicrob Agents Chemother.* 2002;46:1781-1784.

[4] Pfaller MA, Diekema DJ, Messer SA, Boyken L, Hollis RJ. Activities of fluconazole and voriconazole against 1,586 recent clinical isolates of *Candida* species determined by broth microdilution, disk diffusion, and Etest methods: Report from the ARTEMIS Global Antifungal Susceptibility Program, 2001. *J Clin Microbiol.* 2003;41:1440-1446.

[5] Arikan S, Paetznick V, Rex JH. Comparative evaluation of disk diffusion with microdilution assay in susceptibility testing of caspofungin against *Aspergillus* and *Fusarium* isolates. *Antimicrob Agents Chemother.* 2002;46:3084-3087.

[6] Barry AL, Bille J, Brown SD, et al. Quality control limits for fluconazole disk susceptibility tests on Mueller-Hinton agar with glucose and methylene blue. *J Clin Microbiol*. 2003;41:3410-3412.

[7] Matar MJ, Ostrosky-Zeichner L, Paetznick VL, Rodriguez JR, Chen E, Rex JH. Correlation between E-test, disk diffusion, and microdilution methods for antifungal susceptibility testing of fluconazole and voriconazole. *Antimicrob Agents Chemother.* 2003;47:1647-1651.

[8] Rex JH, Pfaller MA, Galgiani JN, et al. Subcommittee on Antifungal Susceptibility Testing of the National Committee of Clinical Laboratory Standards. Development of interpretive breakpoints for antifungal susceptibility testing: Conceptual framework and analysis of *in vitro-in vivo* correlation data for fluconazole, itraconazole, and *Candida* infections. *Clin Infect Dis.* 1997;24:235-247.

[9] Pasarell L, McGinnis MR. Viability of fungal cultures maintained at -70 °C. *J Clin Microbiol.* 1992;30:1000-1004.

[10] Espinol-Ingroff A, Montero D, Martin-Mazuelos E. Long-term preservation of fungal isolates in commercially prepared cryogenic Microbank vials. *J Clin Microbiolo*. 2004;42:1257-1259.

Appendix A. Preparation of Mueller-Hinton Agar + 2% Glucose and 0.5 µg/mL Methylene Blue Dye

The medium can be prepared and poured as the complete media with supplements (A1) *or* the supplements can be added to commercially prepared Mueller-Hinton agar plates (A2). Using the latter technique enables the use of routine Mueller-Hinton agar plates from the bacteriology laboratory.

A1. Preparation of Supplemented Mueller-Hinton Agar:

(1) Mueller-Hinton agar should be prepared from a commercially available dehydrated Mueller-Hinton agar base according to the manufacturer's instructions.

(2) Dissolve 0.1 gram of methylene blue dye in 20 mL of distilled water and warm gently to dissolve. Do not overheat. Add 100 µL of this solution per liter of agar suspension.

(3) Add 20 grams of glucose per liter of agar suspension.

(4) Autoclave as directed by manufacturer's instructions.

(5) Immediately after autoclaving, allow the agar solution to cool in a 45 to 50 °C water bath.

(6) Pour the freshly prepared and cooled medium into plastic, flat-bottomed petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm. This corresponds to 67 to 70 mL of medium for plates with diameters of 150 mm and 28 to 30 mL for plates with a diameter of 100 mm.

(7) The agar medium should be allowed to cool to room temperature and, unless the plate is used on the same day of preparation, stored at refrigerator temperature (2 to 8 °C). The agar medium should have a pH between 7.2 and 7.4 at room temperature (see NCCLS document M7—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*).

(8) Plates should be used within seven days after preparation unless adequate precautions such as wrapping in plastic have been taken to minimize drying of the agar.

(9) A representative sample of each batch of plates should be examined for sterility by incubating at 30 to 35 °C for 24 hours or longer. Plates should undergo quality control testing as per Section 10.

A2. Glucose-Methylene Blue (GMB) Supplementation of Commercially Prepared Mueller-Hinton Agar:

(1) Commercially prepared Mueller-Hinton agar plates can be obtained from several manufacturers. These are the same plates utilized by the bacteriology laboratory for performing Kirby-Bauer disk diffusion tests on bacteria.

(2) Dissolve 0.1 gram of methylene blue dye to 20 mL of distilled water and warm gently to dissolve. Do not overheat. Add 100 µL of this solution per liter of agar suspension.

(3) Prepare a 0.4 g/mL stock solution of glucose by dissolving 40 grams of glucose in 100 mL of distilled water. Heat gently and mix to dissolve.

Appendix A. (Continued)

(4) Add 200 µL of the methylene blue dye stock solution (see (2) above) to 100 mL of the glucose stock solution (see (3) above) to make a GMB stock with a final concentration of 40% glucose and 10 µg/mL of methylene blue dye.

(5) Dispense GMB stock solution in bottles or vials containing 3.5 mL aliquots for 150-mm plates or 1.5 mL aliquots for 90- to 100-mm plates.

(6) Autoclave for 15 minutes at 121 °C followed by slow exhaust.

(7) Store at room temperature and handle aseptically. Do not refrigerate, as this may cause precipitation. A one-year shelf life is generally assumed.

(8) Pour 3.5 mL of the GMB supplement onto the surface of a 150-mm plate with a 70 mL fill *or* 1.5 mL onto a 90- to 100-mm plate with a 30 mL fill. (The actual volume of GMB supplement added may vary slightly depending upon the volume of media plated by the manufacturer. Check with commercially prepared media manufacturers for the exact volume of media used.)

(9) Tilt the plate to spread the supplement evenly. A sterile spreader or sterile bent glass rod can be used to spread the solution evenly across the surface of the agar plate.

(10) Allow the GMB solution to *completely* absorb before inoculating the plate. This requires the plates to be held from 4 to 24 hours prior to use. The plates can be dried at room temperature, incubator temperature, or stored at refrigerated temperature until used. Plates should be used within seven days after preparation unless adequate precautions such as wrapping in plastic have been taken to minimize drying of the agar.

Appendix B. McFarland 0.5 Barium Sulfate Turbidity Standard

To standardize the inoculum density, a $BaSO_4$ turbidity standard is used (0.5 McFarland Standard).

The procedure consists of the following steps:

(1) Prepare this turbidity standard by adding 0.5 mL of 0.048 mol/L $BaCl_2$ (1.175% w/v $BaCl_2 \cdot H_2O$) to 99.5 mL of 0.18 mol/L H_2SO_4 (1% v/v) with constant stirring to maintain a suspension.

(2) Verify the correct density of the turbidity standard by using a spectrophotometer with a 1-cm light path and matched cuvette to determine the absorbance. The absorbance at 625 nm should be 0.08 to 0.10 for the 0.5 McFarland standard.

(3) Distribute 4 to 6 mL into screw-cap tubes of the same size as those used in growing or diluting the broth culture inoculum.

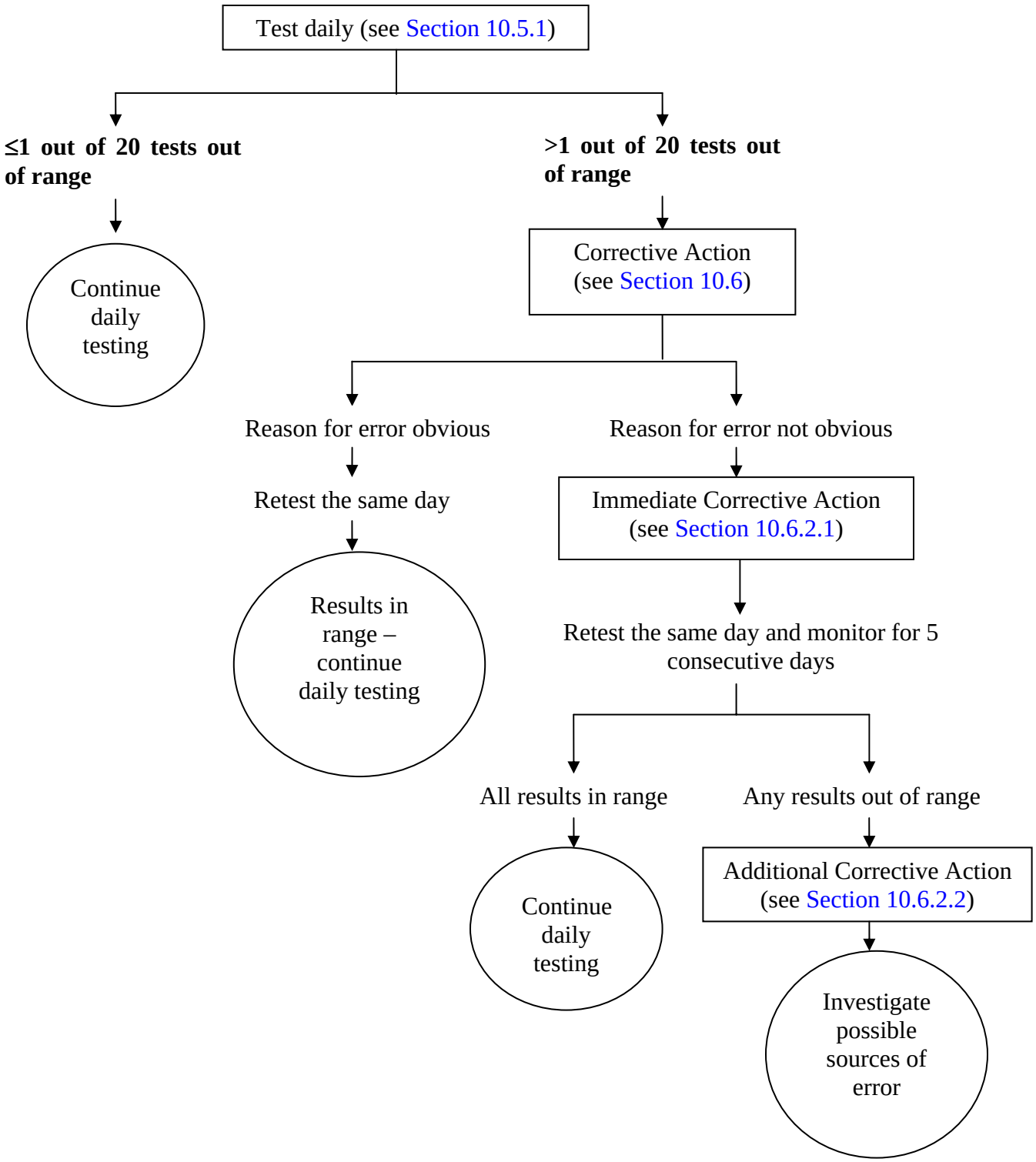
(4) Tightly seal these tubes and store them in the dark at room temperature.

(5) Vigorously agitate this turbidity standard on a mechanical vortex mixer just before use.

(6) The barium sulfate standards should be replaced or their densities verified monthly.

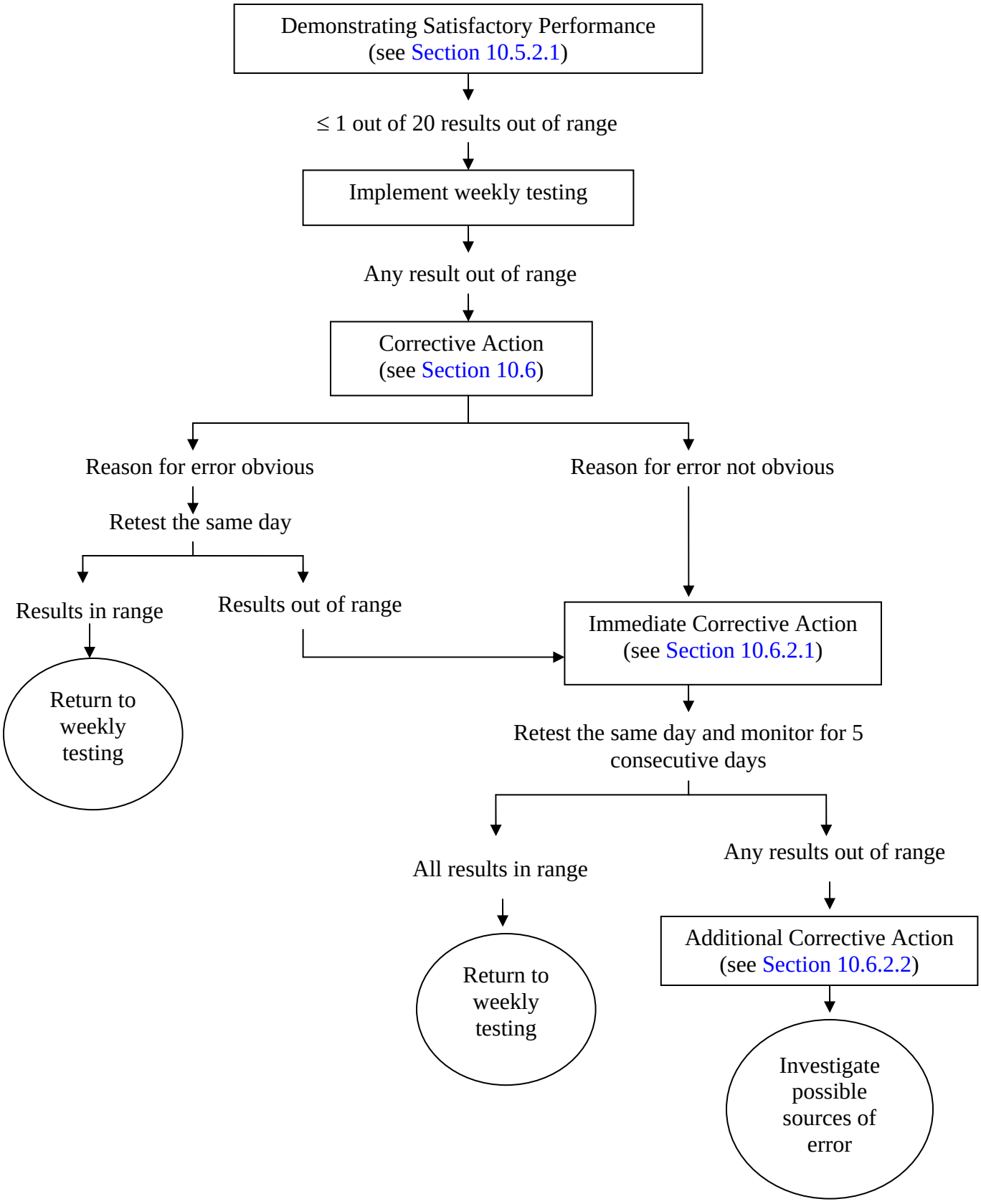
Appendix C. Quality Control Protocol Flow Charts

Disk Diffusion Daily Quality Control Testing Protocol



Appendix C. (Continued)

Disk Diffusion Weekly Quality Control Testing Protocol



NCCLS consensus procedures include an appeals process that is described in detail in Section 8 of the Administrative Procedures. For further information, contact the Executive Offices or visit our website at www.nccls.org.

Summary of Comments and Working Group Responses

M44-P: *Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Proposed Guideline*

Section 5, Selection of Antimicrobial Agent Disks for Routine Testing and Reporting

1. Section 5, "Selection of Antimicrobial Agent Disks for Routine Testing and Reporting," states that disks can be purchased from a variety of commercial sources. Antimicrobial disks are regulated under 21 CFR 430, 431, 460 during the drug approval process by the Center for Drug Evaluation and Research (CDER). Interpretive criteria and quality control procedures are based on those breakpoints established by the FDA (CDER) during the antimicrobial drug review. The Office of *In-Vitro* Diagnostic Device Evaluation and Safety (OIVD) performs only a labeling review of these devices because the scientific evaluation of these disks is complete during the review process of the antimicrobial drug. This procedure has not been done for antifungal drugs; therefore, there are no commercial disks that have been FDA cleared for antifungal testing. Section 7.1.3 also refers to commercially prepared paper disks.

- **The sentence regarding the purchase of disks has been deleted from Section 5**.

Section 5.2, Number of Agents Tested

2. In Section 5.2, the term "limited" is vague. Could you provide a range that qualifies as "limited" (e.g., 3 to 5 antifungals)?

- **A precise number of antimicrobial agents cannot be given since this will vary over time.**

Section 8.3, Application of Disks to Inoculated Agar Plates

3. In Section 8.3, why would you refer to placing 12 disks on a plate when there is only one drug included in Table 1?

- **The subcommittee expects to expand Table 1 in future editions of the document.**

Section 9.1, Zone Diameter Interpretive Standards

4. In Section 9.1, a reference or primary data should be provided that demonstrates how the breakpoints were determined.

- **Three references have been added to this section as suggested.**

Table 2, Recommended Quality Control Zone Diameter (mm) Ranges

5. In Table 2, what is the purpose of the QC for voriconazole when there are no interpretive criteria?

- **These data are needed for ongoing work that seeks to develop interpretive criteria for this compound.**

General

6. Why are there no interpretive standards for itraconazole for ATCC strain 41403?

- **There are no studies at this time for the antimicrobial agent/organism combination referenced.**

NOTES

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:

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

NCCLS document M44-A addresses the quality system essentials (QSEs) indicated by an "X." For a description of the other NCCLS documents listed in the grid, please refer to the Related NCCLS Publications section on the next page.

Documents & Records	Organization	Personnel	Equipment	Purchasing & Inventory	Process Control	Information Management	Occurrence Management	Assessment	Process Improvement	Service & Satisfaction	Facilities & Safety
					X M6 M23						

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: preanalytic, analytic, and postanalytic. All clinical laboratories follow these processes to deliver the laboratory's services, namely quality laboratory information.

NCCLS document M44-A addresses the clinical laboratory path of workflow steps indicated by an "X." For a description of the other NCCLS documents listed in the grid, please refer to the Related NCCLS Publications section on the next page.

Preanalytic					**Analytic**		**Postanalytic**	
Patient Assessment	Test Request	Specimen Collection	Specimen Transport	Specimen Receipt	Testing Review	Laboratory Interpretation	Results Report	Post-test Specimen Management
					X M2 M6 M27 M38	X M2 M6 M27 M38	X M2 M6 M27 M38	M27 M38

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

Related NCCLS Publications*

M2-A8 **Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Eighth Edition (2003).** This standard contains updated recommended techniques, interpretive criteria, and quality control parameters for disk susceptibility testing. This document is complete with disk susceptibility testing tables updated for 2003.

M6-A **Protocols for Evaluating Dehydrated Mueller-Hinton Agar; Approved Standard (1996).** This standard contains procedures for evaluating production lots of Mueller-Hinton agar, and for the development and application of reference media.

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 antimicrobial agents.

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

M38-A **Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard (2002).** 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.

* Proposed- and tentative-level documents are being advanced through the NCCLS consensus process; therefore, readers should refer to the most recent editions.

NOTES

NOTES

Active Membership
(as of 1 April 2004)

Sustaining Members

Abbott Laboratories
American Association for Clinical Chemistry
Bayer Corporation
BD
Beckman Coulter, Inc.
bioMérieux, Inc.
CLMA
College of American Pathologists
GlaxoSmithKline
Ortho-Clinical Diagnostics, Inc.
Pfizer Inc
Roche Diagnostics, Inc.

Professional Members

American Academy of Family Physicians
American Association for Clinical Chemistry
American Association for Respiratory Care
American Chemical Society
American Medical Technologists
American Society for Clinical Laboratory Science
American Society for Microbiology
American Society of Hematology
American Type Culture Collection, Inc.
Asociacion Mexicana de Bioquimica Clinica A.C.
Assn. of Public Health Laboratories
Assoc. Micro. Clinici Italiani-A.M.C.L.I.
British Society for Antimicrobial Chemotherapy
Canadian Society for Medical Laboratory Science - Société Canadienne de Science de Laboratoire Médical
Canadian Standards Association
Clinical Laboratory Management Association
COLA
College of American Pathologists
College of Medical Laboratory Technologists of Ontario
College of Physicians and Surgeons of Saskatchewan
ESCMID
International Council for Standardization in Haematology
International Federation of Biomedical Laboratory Science
International Federation of Clinical Chemistry
Italian Society of Clinical Biochemistry and Clinical Molecular Biology
Japan Society of Clinical Chemistry
Japanese Committee for Clinical Laboratory Standards
Joint Commission on Accreditation of Healthcare Organizations
National Academy of Clinical Biochemistry
National Association of Testing Authorities - Australia
National Society for Histotechnology, Inc.
Ontario Medical Association Quality Management Program-Laboratory Service
RCPA Quality Assurance Programs PTY Limited
Sociedad Espanola de Bioquimica Clinica y Patologia Molecular
Sociedade Brasileira de Analises Clinicas
Taiwanese Committee for Clinical Laboratory Standards (TCCLS)
Turkish Society of Microbiology

Government Members

Armed Forces Institute of Pathology
Association of Public Health Laboratories
BC Centre for Disease Control
Centers for Disease Control and Prevention
Centers for Medicare & Medicaid Services
Centers for Medicare & Medicaid Services/CLIA Program
Chinese Committee for Clinical Laboratory Standards
Commonwealth of Pennsylvania Bureau of Laboratories
Department of Veterans Affairs
Deutsches Institut für Normung (DIN)
FDA Center for Devices and Radiological Health
FDA Center for Veterinary Medicine
FDA Division of Anti-Infective Drug Products
Iowa State Hygienic Laboratory
Massachusetts Department of Public Health Laboratories
National Center of Infectious and Parasitic Diseases (Bulgaria)
National Health Laboratory Service (South Africa)
National Institute of Standards and Technology
New York State Department of Health
Ontario Ministry of Health
Pennsylvania Dept. of Health
Saskatchewan Health-Provincial Laboratory
Scientific Institute of Public Health; Belgium Ministry of Social Affairs, Public Health and the Environment
Swedish Institute for Infectious Disease Control

Industry Members

AB Biodisk
Abbott Laboratories
Abbott Laboratories, MediSense Products
Acrometrix Corporation
Advancis Pharmaceutical Corporation
Alifax S.P.A.
Ammirati Regulatory Consulting
A/S ROSCO
AstraZeneca Pharmaceuticals
Aventis
Axis-Shield PoC AS
Bayer Corporation - Elkhart, IN
Bayer Corporation - Tarrytown, NY
Bayer Corporation - West Haven, CT
BD
BD Consumer Products
BD Diagnostic Systems
BD Thailand Ltd.
BD VACUTAINER Systems
Beckman Coulter, Inc.
Beckman Coulter K.K. (Japan)
Bio-Development SRL
Bio-Inova Life Sciences International
Biomedia Laboratories SDN BHD
bioMérieux, Inc. (MO)
Biometrology Consultants
Bio-Rad Laboratories, Inc.
Bio-Rad Laboratories, Inc. – France
BioVeris Corporation
Blaine Healthcare Associates, Inc.
Bristol-Myers Squibb Company
Canadian External Quality Assessment Laboratory
Cepheid
Chiron Corporation
ChromaVision Medical Systems, Inc.
Clinical Micro Sensors
The Clinical Microbiology Institute
Cognigen
Copan Diagnostics Inc.
Cosmetic Ingredient Review
Cubist Pharmaceuticals
Dade Behring Inc. - Cupertino, CA
Dade Behring Inc. - Deerfield, IL
Dade Behring Inc. - Glasgow, DE
Dade Behring Inc. - Marburg, Germany
Dade Behring Inc. - Sacramento, CA
David G. Rhoads Associates, Inc.
Diagnostic Products Corporation
Digene Corporation
Eiken Chemical Company, Ltd.
Elanco Animal Health
Electa Lab s.r.l.
Enterprise Analysis Corporation
EXPERTech Associates, Inc.
F. Hoffman-La Roche AG
Fort Dodge Animal Health
Gen-Probe
GenVault
GlaxoSmithKline
Greiner Bio-One Inc.
Immunicon Corporation
ImmunoSite, Inc.
Instrumentation Laboratory
International Technidyne Corporation
I-STAT Corporation
Johnson & Johnson Pharmaceutical Research & Development, L.L.C.
LAB-Interlink, Inc.
Laboratory Specialists, Inc.
Labtest Diagnostica S.A.
LifeScan, Inc. (a Johnson & Johnson Company)
LUZ, Inc.
Machaon Diagnostics
Medical Device Consultants, Inc.
Merck & Company, Inc.
Minigrip/Zip-Pak
mvi Sciences (MA)
Nippon Becton Dickinson Co., Ltd.
Nissui Pharmaceutical Co., Ltd.
Norfolk Associates, Inc.
Novartis Pharmaceutical Corporation
Olympus America, Inc.
Ortho-Clinical Diagnostics, Inc. (Rochester, NY)
Ortho-McNeil Pharmaceutical (Raritan, NJ)
Oxoid Inc.
Paratek Pharmaceuticals
Pfizer Inc
Pfizer Inc - Kalamazoo, MI
Pfizer Italia Srl
Powers Consulting Services
Premier Inc.
Procter & Gamble Pharmaceuticals, Inc.
QSE Consulting
Quintiles, Inc.
Radiometer America, Inc.
Radiometer Medical A/S
Replidyne
Roche Diagnostics GmbH
Roche Diagnostics, Inc.
Roche Laboratories (Div. Hoffmann-La Roche Inc.)
Sarstedt, Inc.
Schering Corporation
Schleicher & Schuell, Inc.
Second Opinion
Seraphim Life Sciences Consulting LLC
Streck Laboratories, Inc.
SYN X Pharma Inc.
Sysmex Corporation (Japan)
Sysmex Corporation (Long Grove, IL)
Theravance Inc.
THYMED GmbH
Transasia Engineers
Trek Diagnostic Systems, Inc.
Tyco Kendall Healthcare
Vetoquinol S.A.
Vicuron Pharmaceuticals Inc.
Vysis, Inc.
Wyeth Research
XDX, Inc.
Xyletech Systems, Inc.
YD Consultant
YD Diagnostics (Seoul, Korea)

Trade Associations

AdvaMed
Japan Association of Clinical Reagents Industries (Tokyo, Japan)

Associate Active Members

31st Medical Group/SGSL (APO, AE)
Academisch Ziekenhuis -VUB (Belgium)
Alfred I. du Pont Hospital for Children (DE)
Allina Health System (MN)
American University of Beirut Medical Center (NY)
Anne Arundel Medical Center (MD)
Antwerp University Hospital (Belgium)
Arkansas Department of Health
ARUP at University Hospital (UT)
Associated Regional & University Pathologists (UT)
Atlantic Health System (NJ)
Aurora Consolidated Laboratories (WI)
AZ Sint-Jan (Belgium)
Azienda Ospedale Di Lecco (Italy)
Barnes-Jewish Hospital (MO)
Baxter Regional Medical Center (AR)
Baystate Medical Center (MA)
Bbaguas Duzen Laboratories (Turkey)
BC Biomedical Laboratories (Surrey, BC, Canada)
Bermuda Hospitals Board
Boulder Community Hospital (CO)
Brazosport Memorial Hospital (TX)
Brooks Air Force Base (TX)
Broward General Medical Center (FL)
Cadham Provincial Laboratory (Winnipeg, MB, Canada)
Calgary Laboratory Services (Calgary, AB, Canada)
Cape Breton Healthcare Complex (Nova Scotia, Canada)
Carilion Consolidated Laboratory (VA)
Carolinas Medical Center (NC)
Cathay General Hospital (Taiwan)
Central Texas Veterans Health Care System
Centro Diagnostico Italiano (Milano, Italy)
Champlain Valley Physicians Hospital (NY)
Chang Gung Memorial Hospital (Taiwan)
Changi General Hospital (Singapore)
Children's Hospital (NE)
Children's Hospitals and Clinics (MN)
Children's Hospital Medical Center (Akron, OH)
Children's Hospital of Wisconsin
Children's Medical Center of Dallas (TX)
CHR St. Joseph Warquignies (Belgium)
Christus St. John Hospital (TX)
Clarian Health - Methodist Hospital (IN)
CLSI Laboratories (PA)
Community Hospital of Lancaster (PA)
Community Hospital of the Monterey Peninsula (CA)
CompuNet Clinical Laboratories (OH)
Cook Children's Medical Center (TX)
Cook County Hospital (IL)
Covance Central Laboratory Services (IN)
Creighton University Medical Center (NE)
Danish Veterinary Laboratory (Denmark)
Detroit Health Department (MI)
DFS/CLIA Certification (NC)
Diagnósticos da América S/A (Brazil)
Dr. Everett Chalmers Hospital (New Brunswick, Canada)
Duke University Medical Center (NC)
Dwight David Eisenhower Army Medical Center (GA)
EMH Regional Medical Center (OH)
Emory University Hospital (GA)
Enzo Clinical Labs (NY)
Evangelical Community Hospital (PA)
Fairview-University Medical Center (MN)
Florida Hospital East Orlando
Focus Technologies, Inc. (CA)
Focus Technologies, Inc. (VA)
Foothills Hospital (Calgary, AB, Canada)
Franciscan Shared Laboratory (WI)
Fresno Community Hospital and Medical Center
Frye Regional Medical Center (NC)
Gamma Dynacare Medical Laboratories (ON, Canada)
Geisinger Medical Center (PA)
General Health System (LA)
Grady Memorial Hospital (GA)
Guthrie Clinic Laboratories (PA)
Hagerstown Medical Laboratory (MD)
Hahnemann University Hospital (PA)
Harris Methodist Fort Worth (TX)
Hartford Hospital (CT)
Headwaters Health Authority (Alberta, Canada)
Health Network Lab (PA)
Health Partners Laboratories (VA)

Highlands Regional Medical Center (FL)
Hoag Memorial Hospital Presbyterian (CA)
Holy Cross Hospital (MD)
Hôpital du Sacré-Coeur de Montreal (Montreal, Quebec, Canada)
Hôpital Maisonneuve - Rosemont (Montreal, Canada)
Hôpital Saint-Luc (Montreal, Quebec, Canada)
Hospital Consolidated Laboratories (MI)
Hospital for Sick Children (Toronto, ON, Canada)
Hospital Sousa Martins (Portugal)
Hotel Dieu Grace Hospital (Windsor, ON, Canada)
Huddinge University Hospital (Sweden)
Hunter Area Pathology Service (Australia)
Indiana University
Innova Fairfax Hospital (VA)
Institute of Medical and Veterinary Science (Australia)
International Health Management Associates, Inc. (IL)
Jackson Memorial Hospital (FL)
Jacobi Medical Center (NY)
John C. Lincoln Hospital (AZ)
Johns Hopkins Medical Institutions (MD)
Kadlec Medical Center (WA)
Kaiser Permanente (CA)
Kaiser Permanente (MD)
Kangnam St. Mary's Hospital (Korea)
Kantonsspital (Switzerland)
Kenora-Rainy River Regional Laboratory Program (Ontario, Canada)
Kimball Medical Center (NJ)
King Faisal Specialist Hospital (Saudi Arabia)
LabCorp (NC)
Laboratoire de Santé Publique du Quebec (Canada)
Laboratorio Dr. Echevarne (Spain)
Laboratório Fleury S/C Ltda. (Brazil)
Laboratorio Manlab (Argentina)
Laboratory Corporation of America (NJ)
LAC & USC Healthcare Network (CA)
Lakeland Regional Medical Center (FL)
Landstuhl Regional Medical Center (APO AE)
LeBonheur Children's Medical Center (TN)
Lewis-Gale Medical Center (VA)
L'Hotel-Dieu de Quebec (Canada)
Libero Instituto Univ. Campus BioMedico (Italy)
Loma Linda Mercantile (CA)
Long Beach Memorial Medical Center (CA)
Louisiana State University Medical Center
Maccabi Medical Care and Health Fund (Israel)
Magnolia Regional Health Center (MS)
Maimonides Medical Center (NY)
Marion County Health Department (IN)
Martin Luther King/Drew Medical Center (CA)
Massachusetts General Hospital (Microbiology Laboratory)
MDS Metro Laboratory Services (Burnaby, BC, Canada)
Medical College of Virginia Hospital
Memorial Medical Center (Jefferson Davis Pkwy., New Orleans, LA)
Memorial Medical Center (Napoleon Avenue, New Orleans, LA)
Methodist Hospital (TX)
Michigan Department of Community Health
Mid America Clinical Laboratories, LLC (IN)
Middlesex Hospital (CT)
Monmouth Medical Center (NJ)
Montreal Children's Hospital (Canada)
Montreal General Hospital (Canada)
National Serology Reference Laboratory (Australia)
NB Department of Health & Wellness (New Brunswick, Canada)
The Nebraska Medical Center
New Britain General Hospital (CT)
New England Fertility Institute (CT)
New England Medical Center (MA)
New York University Medical Center
NorDx (ME)
North Carolina State Laboratory of Public Health
North Central Medical Center (TX)
North Shore-Long Island Jewish Health System Laboratories (NY)
North Shore University Hospital (NY)
Northwestern Memorial Hospital (IL)
Ochsner Clinic Foundation (LA)
O.L. Vrouwziekenhuis (Belgium)
Ordre professionnel des technologists médicaux du Québec
Ospedali Riuniti (Italy)
The Ottawa Hospital (Ottawa, ON, Canada)
OU Medical Center (OK)
Our Lady of the Resurrection Medical Center (IL)
Pathology and Cytology Laboratories, Inc. (KY)
Pathology Associates Medical Laboratories (WA)
The Permanente Medical Group (CA)
Piedmont Hospital (GA)
Pocono Medical Center (PA)
Presbyterian Hospital of Dallas (TX)
Providence Health Care (Vancouver, BC, Canada)
Provincial Laboratory for Public Health (Edmonton, AB, Canada)
Queen Elizabeth Hospital (Prince Edward Island, Canada)
Queensland Health Pathology Services (Australia)
Quest Diagnostics Incorporated (CA)
Quintiles Laboratories, Ltd. (GA)
Regions Hospital
Rex Healthcare (NC)
Rhode Island Department of Health Laboratories
Riverside Medical Center (IL)
Riyadh Armed Forces Hospital (Saudi Arabia)
Robert Wood Johnson University Hospital (NJ)
Royal Columbian Hospital (New Westminster, BC, Canada)
Saad Specialist Hospital (Saudi Arabia)
Sahlgrenska Universitetssjukhuset (Sweden)
Saint Mary's Regional Medical Center (NV)
St. Alexius Medical Center (ND)
St. Anthony Hospital (CO)
St. Anthony's Hospital (FL)
St. Barnabas Medical Center (NJ)
St-Eustache Hospital (Quebec, Canada)
St. Francis Medical Center (CA)
St. John Hospital and Medical Center (MI)
St. John's Hospital & Health Center (CA)
St. Joseph Mercy Hospital (MI)
St. Joseph's Hospital-Marshfield Clinic (WI)
St. Joseph's Hospital & Medical Center (AZ)
St. Jude Children's Research Hospital (TN)
St. Mary of the Plains Hospital (TX)
St. Michael's Hospital (Toronto, ON, Canada)
Ste. Justine Hospital (Montreal, PQ, Canada)
Salem Clinic (OR)
San Francisco General Hospital (CA)
Santa Clara Valley Medical Center (CA)
Seoul Nat'l University Hospital (Korea)
Shands at the University of Florida
So. California Permanente Medical Group
South Bend Medical Foundation (IN)
South Western Area Pathology Service (Australia)
Southern Maine Medical Center
Southwest Texas Methodist Hospital
Spartanburg Regional Medical Center (SC)
Specialty Laboratories, Inc. (CA)
State of Connecticut Dept. of Public Health
State of Washington Department of Health
Stony Brook University Hospital (NY)
Stormont-Vail Regional Medical Center (KS)
Sun Health-Boswell Hospital (AZ)
Sunnybrook Health Science Center (ON, Canada)
Swedish Medical Center - Providence Campus (WA)
Temple University Hospital (PA)
Tenet Odessa Regional Hospital (TX)
The Toledo Hospital (OH)
Touro Infirmary (LA)
Tripler Army Medical Center (HI)
Truman Medical Center (MO)
UCLA Medical Center (CA)
UCSF Medical Center (CA)
UNC Hospitals (NC)
Unidad de Patologia Clinica (Mexico)
Union Clinical Laboratory (Taiwan)
Universita Campus Bio-Medico (Italy)
University Hospitals of Cleveland (OH)
University of Alabama-Birmingham Hospital
University of Chicago Hospitals (IL)
University of Colorado Hospital
University of Debrecen Medical Health and Science Center (Hungary)
University of Illinois Medical Center
University of the Ryukyus (Japan)
The University of Texas Medical Branch
The University of the West Indies
University of Virginia Medical Center
University of Washington
UZ-KUL Medical Center (Belgium)
VA (Hampton) Medical Center (VA)
VA (Hines) Medical Center (IL)
VA (Tuskegee) Medical Center (AL)
Valley Children's Hospital (CA)
Vejle Hospital (Denmark)
Virginia Beach General Hospital (VA)
Virginia Regional Medical Center (MN)
ViroMed Laboratories (MN)
Washington Adventist Hospital (MD)
Washoe Medical Center Laboratory (NV)
Waterford Regional Hospital (Ireland)
Wellstar Health System (GA)
West Jefferson Medical Center (LA)
Wilford Hall Medical Center (TX)
William Beaumont Army Medical Center (TX)
William Beaumont Hospital (MI)
William Osler Health Centre (Brampton, ON, Canada)
Winn Army Community Hospital (GA)
Winnipeg Regional Health Authority (Winnipeg, Canada)
Wishard Memorial Hospital (IN)
Yonsei University College of Medicine (Korea)
York Hospital (PA)

NCCLS ▼ 940 West Valley Road ▼ Suite 1400 ▼ Wayne, PA 19087 ▼ USA ▼ PHONE 610.688.0100
FAX 610.688.0700 ▼ E-MAIL: exoffice@nccls.org ▼ WEBSITE: www.nccls.org ▼ ISBN 1-56238-532-1