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Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Approved Guideline

This document provides guidance to clinical microbiology laboratories for standardized susceptibility testing of infrequently isolated or fastidious bacteria that are not presently included in CLSI/NCCLS documents M2, M7, or M11. The tabular information in this document presents the most current information for drug selection, interpretation, and quality control for the infrequently isolated or fastidious bacterial pathogens included in this guideline.

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

CLINICAL AND LABORATORY STANDARDS INSTITUTE ™

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Advancing Quality in Healthcare Testing

Clinical and Laboratory Standards Institute (CLSI, formerly 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. Our process 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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A document is published as a standard, guideline, or committee report.

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The CLSI 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 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 consensus level.

Proposed A 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.

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.

Our 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 CLSI's established consensus procedures. Provisions in CLSI 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 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 document. Address comments to Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087, USA.

VOLUNTEER PARTICIPATION

Healthcare professionals in all specialties are urged to volunteer for participation in CLSI projects. Please contact us at customerservice@clsi.org or +610.688.0100 for additional information on committee participation.

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Abstract

If the susceptibility of a bacterial pathogen to antimicrobial agents cannot be predicted based on the identity of the organism alone, *in vitro* antimicrobial susceptibility testing of the organism isolated from the disease processes is indicated. Susceptibility testing is particularly necessary in those situations where the etiologic agent belongs to a bacterial species for which resistance to commonly used antimicrobial agents has been documented, or could arise.

A variety of laboratory techniques can be used to measure the *in vitro* susceptibility of bacteria to antimicrobial agents. This document describes the standard microdilution and agar disk diffusion methods. It also includes a series of procedures designed to standardize test performance. The performance, applications, and limitations of the current CLSI-recommended methods are described.

The tabular information in this document presents the most current information for drug selection, interpretation, and quality control for the infrequently isolated or fastidious bacterial pathogens included in this guideline. As more information becomes available, changes will be incorporated into future revisions of this document.

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Foreword

This document was developed for the purpose of providing guidance to clinical microbiology laboratories regarding the performance of standardized susceptibility testing, when needed, of infrequently isolated or fastidious bacteria that are not presently included in the most current editions of CLSI/NCCLS documents M2—*Performance Standards for Antimicrobial Disk Susceptibility Tests*, M7—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*, or M11—*Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria*. Some of the organisms included herein are aerobic gram-negative bacilli that are not members of the family *Enterobacteriaceae*, but may be tested by the standard CLSI broth microdilution or disk diffusion methods in the same manner as the much more common *Enterobacteriaceae* isolates. Some aerobic gram-positive cocci and bacilli that are encountered periodically by clinical laboratories can likewise be tested reliably by the standard CLSI MIC or disk diffusion test methods in a manner analogous to *Staphylococcus* or *Enterococcus* spp. In addition, several genera of fastidious gram-positive and gram-negative bacteria can be tested in the same manner as the streptococci, using blood-supplemented Mueller-Hinton media. For the purpose of this document, the term *fastidious* is used to describe bacteria that require media supplemented with blood or blood components and that possibly need an atmosphere other than ambient air (e.g., with 5% CO_2) for acceptable growth. Because the standard CLSI media, reagents, and procedures can be used to test the organisms included in this guideline, the quality control procedures, strains, and acceptable zone diameter and MIC limits that have been established through previous rigorous studies can be utilized for tests with the less common organisms that are included in this document. The working group used a thorough search of the published literature in conjunction with the clinical experience of the members to apply or adapt interpretive criteria or breakpoints from other organisms that could best be applied to the interpretation of tests of the less common organisms in this document. Users of the guideline should be aware that the very extensive microbiological, clinical, and pharmacodynamic databases normally employed for setting breakpoints by CLSI did not exist for the collection of "orphan" organisms described in this document.

It is important for users of M45-A to recognize that commercial susceptibility testing devices are not addressed in this guideline. The methods described herein are generic reference procedures that can be used for routine susceptibility testing by clinical laboratories, or that can be used by clinical laboratories to evaluate commercial devices for possible routine use. Results generated by the CLSI reference methods are used by the United States Food and Drug Administration to evaluate the performance of commercial systems before clearance is given for marketing in the United States. Clearance by the FDA indicates that the agency concludes that commercial devices provide susceptibility results that are substantially equivalent to results generated using the CLSI reference methods for the organisms and antimicrobial agents described in the manufacturer's approved package insert. Some laboratories could find that a commercial dilution, antibiotic gradient, colorimetric, turbidimetric, fluorometric, or other method is suitable for selective or routine use.

Key Words

Agar dilution, antimicrobial agent, antimicrobial susceptibility, broth dilution, disk diffusion, microdilution, minimal inhibitory concentration (MIC), susceptibility testing

CLSI Subcommittee on Antimicrobial Susceptibility Testing Mission Statement

The CLSI Subcommittee on Antimicrobial Susceptibility Testing is composed of representatives from the professions, government, and industry, including microbiology laboratories, government agencies, healthcare providers and educators, and pharmaceutical and diagnostic microbiology industries. Using the CLSI voluntary consensus process, the subcommittee develops standards that promote accurate antimicrobial susceptibility testing and appropriate reporting.

The mission of the CLSI Subcommittee on Antimicrobial Susceptibility Testing is to:

- develop standard reference methods for antimicrobial susceptibility tests;
- provide quality control parameters for standard test methods;
- establish interpretive criteria for the results of standard antimicrobial susceptibility tests;
- provide suggestions for testing and reporting strategies that are clinically relevant and cost-effective;
- continually refine standards and optimize the detection of emerging resistance mechanisms through the development of new or revised methods, interpretive criteria, and quality control parameters;
- educate users through multimedia communication of standards and guidelines; and
- foster a dialogue with users of these methods and those who apply them.

The ultimate purpose of the subcommittee's mission is to provide useful information to enable laboratories to assist the clinician in the selection of appropriate antimicrobial therapy for patient care. The standards and guidelines are meant to be comprehensive and to include all antimicrobial agents for which the data meet established CLSI guidelines. The values that guide this mission are quality, accuracy, fairness, timeliness, teamwork, consensus, and trust.

Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Approved Guideline

1 Scope

CLSI documents M2—*Performance Standards for Antimicrobial Disk Susceptibility Tests* and M7—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically* describe standardized methods for antimicrobial susceptibility testing of common aerobic bacteria, including some fastidious organisms or potential agents of bioterrorism. However, there are a number of less frequently encountered or fastidious bacteria that are not addressed in M2, M7, and M100—*Performance Standards for Antimicrobial Susceptibility Testing*. Some of these are organisms that may cause serious infections (e.g., infective endocarditis), infections associated with trauma and environmental contamination, or device-associated infections in immunocompromised or postsurgical patients (e.g., intravascular catheters, implanted devices, central nervous system shunts). Key gram-positive organisms include *Corynebacterium* spp., *Bacillus* spp. (not *B. anthracis*), and several genera that have intrinsic vancomycin resistance. Nonfastidious gram-negative bacteria include *Aeromonas* spp., *Plesiomonas* spp., *Vibrio* spp., and *Moraxella catarrhalis.*

The fastidious gram-negative bacilli include the HACEK group, *Campylobacter*, and *Pasteurella* spp. Organisms considered to be members of the HACEK group are the Aphrophilus cluster of the genus *Haemophilus* (i.e., *H. aphrophilus, H. paraphrophilus, H. segnis), Actinobacillus actinomycetemcomitans*, *Cardiobacterium* spp., *Eikenella corrodens*, and *Kingella* spp. *Capnocytophaga* spp. are outside the scope of this document because of the lack of suitable methods for broth dilution or disk diffusion susceptibility testing. The *Capnocytophaga* spp. frequently produce β-lactamase, but they are generally susceptible to β-lactam/β-lactamase inhibitor combinations, clindamycin, imipenem, and linezolid.

Fastidious gram-positive bacteria that may cause endocarditis include *Abiotrophia* spp. and *Granulicatella* spp. Acquired antimicrobial resistance mechanisms have been reported in many of these organisms, and the medical literature includes descriptions of susceptibility results derived from use of standard CLSI methods or certain nonstandard procedures.

Because infections due to organisms addressed in M45 occur less frequently than many of the organisms presently covered in CLSI documents M2 and M7, and the fact that many of the antimicrobial agents of interest have been marketed for a number of years, it is not reasonable to expect the intensive CLSI/NCCLS document M23-specified studies (*Development of* In Vitro *Susceptibility Testing Criteria and Quality Control Parameters*) to be conducted on this special group of organisms. Instead, the goal of this document is to propose test conditions and interpretive criteria based upon a careful review of published microbiological data (distributions of MICs), and the extant clinical literature regarding therapy for these organisms, and in a few instances, a review of existing pharmacokinetic data on the drugs of interest. In some cases, limited *in vitro* testing was performed. It is hoped that this CLSI guideline will assist clinical microbiology laboratories in determining an approach for testing these unusual organisms that is relevant to their individual practice settings.

2 Introduction

CLSI documents M2 and M7 describe reference and standardized methods for antimicrobial susceptibility testing of common, rapidly growing aerobic bacteria, including staphylococci, enterococci, members of the *Enterobacteriaceae*, *Pseudomonas* spp., and *Acinetobacter* spp., *Burkholderia cepacia*, and *Stenotrophomonas maltophilia* (in addition to a few other nonglucose-fermentative gram-negative bacilli). These documents also include standard susceptibility testing methods, quality control values, and specific breakpoints for several fastidious bacterial species, including *Haemophilus influenzae, Neisseria*

