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Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Eighth Edition

This document addresses reference methods for the determination of minimal inhibitory concentrations (MICs) of aerobic bacteria by broth macrodilution, broth microdilution, and agar dilution.

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

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Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Eighth Edition

Matthew A. Wikler, MD, MBA, FIDSA
Franklin R. Cockerill, III, MD
Karen Bush, PhD
Michael N. Dudley, PharmD, FIDSA
George M. Eliopoulos, MD
Dwight J. Hardy, PhD
David W. Hecht, MD
Janet F. Hindler, MCLS, MT(ASCP)
Jean B. Patel, PhD, D(ABMM)
Mair Powell, MD, FRCP, FRCPath
John D. Turnidge, MD
Melvin P. Weinstein, MD
Barbara L. Zimmer, PhD

Mary Jane Ferraro, PhD, MPH
Jana M. Swenson, MMSc

Abstract

Susceptibility testing is indicated for any organism that contributes to an infectious process warranting antimicrobial chemotherapy, if its susceptibility cannot be reliably predicted from knowledge of the organism's identity. Susceptibility tests are most often indicated when the causative organism is thought to belong to a species capable of exhibiting resistance to commonly used antimicrobial agents.

A variety of laboratory methods can be used to measure the *in vitro* susceptibility of bacteria to antimicrobial agents. This document describes standard broth dilution (macrodilution and microdilution [the microdilution method described in M07 is the same methodology outlined in ISO 20776-1])[1] and agar dilution techniques, and it includes a series of procedures to standardize the way the tests are performed. The performance, applications, and limitations of the current CLSI-recommended methods are also described.

The supplemental information (M100 tables) presented with this standard represents the most current information for drug selection, interpretation, and quality control using the procedures standardized in M07. These tables, as in previous years, have been updated and should replace tables published in earlier years. Changes in the tables since the previous edition (M100-S18) appear in boldface type and are also summarized in the front of the document.

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Foreword

In this 2009 revision of CLSI document M07, several sections have been added or revised as outlined in the Summary of Changes. The latest version of the M100 tables (M100-S19) published as an annual volume is made available with this document to ensure that users are aware of the latest subcommittee guidelines related to both methods and the tabular information normally presented in the annual tables. M100-S19 will be updated during subcommittee meetings in 2009 and published again as a separate document in January 2010.

Many other editorial and procedural changes in this edition of M07 resulted from meetings of the Subcommittee on Antimicrobial Susceptibility Testing since 2006. Specific changes for the M100 tables are summarized at the beginning of the M100-S19 document. The most important changes in the M07 document are summarized below.

It has been an honor to serve as Chairholder of the Subcommittee on Antimicrobial Susceptibility Testing during the last three years. Many members of the subcommittee, which now numbers more than 180 volunteers including members, advisors, and observers, have been indispensable in the preparation of these documents. In addition, I would like to thank the chairholders of the working groups of the Subcommittee on Antimicrobial Susceptibility Testing for their valuable contributions during the last three years. They include Jana Swenson (Text and Table Revision and *Acinetobacter* Working Groups); Frank Cockerill (Agents of Bioterrorism Working Group); Sharon Cullen and Steve Brown (Quality Control Working Group); Dwight Hardy (*Stenotrophomonas* and *Burkholderia* Working Group); George Eliopoulos (M23—Development of *In Vitro* Susceptibility Testing Criteria and Quality Control Parameters Working Group); John McGowan (Communications Working Group); Janet Hindler (M39—Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data Working Group); David Hecht (M11—Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria Working Group); Fred Tenover (Staphylococci Working Group); Mike Dudley (*Enterobacteriaceae* Working Group); Jim Jorgensen (M45—Methods for Antimicrobial Dilution and Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria Working Group); and Barth Reller (Table 1 Working Group).

Matthew A. Wikler, MD, MBA, FIDSA
Chairholder, Subcommittee on Antimicrobial Susceptibility Testing

Summary of Major Changes in This Document

Summary of CLSI Processes for Establishing Interpretive Criteria and QC Ranges
Added information on the process utilized by the Subcommittee on Antimicrobial Susceptibility Testing and the data that are required to establish interpretive criteria, quality control parameters for updating this document.
Added URL for locating minutes from Subcommittee on Antimicrobial Susceptibility Testing meetings

CLSI Reference Methods vs Commercial Methods and CLSI vs FDA Breakpoints (interpretive criteria)
New heading for text box.

Section 4.1, Definitions
Added definitions for D-zone test, quality assurance (QA), nonsusceptible, and saline.

Section 4.2, Abbreviations/Acronyms
Added an Abbreviations/Acronyms section.

Section 6.2.2.5, Macrolides
Listed the subgroups of antimicrobials for the macrolide group.

Summary of Major Changes in This Document (Continued)

Section 6.2.2.7, Tetracyclines
Added information on tigecycline, a glycylcycline.

Moved instructions for media and reagent preparation to Appendix B, which include those from:
Section 8.1, Turbidity standard for inoculum preparation
Section 9.1.1, Mueller-Hinton agar
Section 10.1, Mueller-Hinton broth
Section 11.1, *Haemophilus* Test Medium (HTM)
Section 11.2, GC agar
Section 11.3, Mueller-Hinton agar supplemented with 5% sheep blood
Section 11.4, CAMHB with 2.5% to 5% lysed horse blood

Section 11.3, *Neisseria meningitidis*
Added cautionary statement for performing susceptibility testing in a biological safety cabinet.

Section 12.1.3.1, Methods for Detection of Reduced Susceptibility to Vancomycin
Added table summarizing the various methods to detect levels of vancomycin susceptibility in *S. aureus*.

Section 12.1.3.3, Heteroresistant Vancomycin-Intermediate *Staphylococcus aureus* (hVISA)
Added discussion of hVISA.

Section 12.1.5, Mupirocin Resistance
Added method for detecting and reporting high-level mupirocin resistance (ie, MICs ≥ 512 µg/mL) in *S. aureus*.

Section 12.3, β-Lactamase-Mediated Resistance in Gram-Negative Bacilli
Added table showing the molecular classification of β-lactamases and discussion of plasmid-encoded β-lactamases, *Klebsiella pneumoniae* carbapenemase (KPC) carbapenemases, AmpC β-lactamases, and metallo-β-lactamases.

Section 16.2, Quality Control Responsibilities
Added new section outlining the quality control responsibilities of both manufacturers and users.

Section 16.3, Selection of Quality Control Strains for Quality Control and Quality Assurance
Expanded section on using, selecting, and obtaining quality control strains and defined QC strain and supplemental QC strain.

Section 16.7.1, Daily Testing
Clarified consecutive results as consecutive test days.

Section 16.9.1, Out-of-Control Result Due to Identifiable Errors
Expanded on the possible causes for out-of-control results and strategy for corrective action.

Section 16.9.2, Out-of-Control Result With No Error Identified
Expanded on the possible causes for out-of-control results and strategy for corrective action.

Summary of Major Changes in This Document (Continued)

Section 16.12, Other Control Procedures
Added section outlining inoculum control and end-point interpretation control.

Appendix B, Preparation of Supplements, Media, and Reagents
Added new appendix listing media and reagent preparation instructions.

Appendix C, Conditions for Dilution Antimicrobial Susceptibility Tests
Added new appendix providing medium, incubation temperature, incubation time, and minimal quality control for organisms addressed in this document and listed in M100 Table 2 series.

Appendix D, Quality Control Strains for Antimicrobial Susceptibility Tests
New appendix providing quality control organism characteristics.

Appendix E, Quality Control Strain Maintenance
Added new appendix providing steps for quality control strain maintenance.

Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Eighth Edition

1 Scope

This document describes the standard broth (macrodilution and microdilution) and agar dilution methods used to determine the *in vitro* susceptibility of bacteria that grow aerobically. It addresses preparation of broth and agar dilution tests, testing conditions (including inoculum preparation and standardization, incubation time, and incubation temperature), reporting of minimal inhibitory concentration (MIC) results, quality control (QC) procedures, and limitations of the dilution test methods. To assist the clinical laboratory, suggestions are provided on the selection of antimicrobial agents for routine testing and reporting. Standards for testing the *in vitro* susceptibility of bacteria that grow aerobically utilizing the antimicrobial disk susceptibility testing method are found in CLSI document M02.[3] Standards for testing the *in vitro* susceptibility of bacteria that grow anaerobically are found in CLSI document M11.[4] Guidelines for standardized susceptibility testing of infrequently isolated or fastidious bacteria that are not included in CLSI documents M02,[3] M07, or M11[4] are available in CLSI document M45.[5]

2 Introduction

Either broth or agar dilution methods may be used to measure quantitatively the *in vitro* activity of an antimicrobial agent against a given bacterial isolate. To perform the tests, a series of tubes or plates is prepared with a broth or agar medium to which various concentrations of the antimicrobial agents are added. The tubes or plates are then inoculated with a standardized suspension of the test organism. After incubation at 35 ± 2 °C, the tests are examined and the MIC is determined. The final result is significantly influenced by methodology, which must be carefully controlled if reproducible results (intralaboratory and interlaboratory) are to be achieved.

This document describes reference standard broth dilution (macrodilution and microdilution) and agar dilution methods. The basics of these methods are derived, in large part, from information generated by the International Collaborative Study.[6] Although these methods are standard reference methods, some are sufficiently practical for routine use in both clinical laboratories and research laboratories.

Commercial systems based primarily, or in part, on certain of these methods are available and may provide essentially equivalent results to the CLSI methods described here. The US Food and Drug Administration (FDA) is responsible for the approval of commercial devices used in the United States. CLSI does not approve or endorse commercial products or devices.

The methods described in this document are intended primarily for testing commonly isolated aerobic or facultative bacteria that grow well after overnight incubation in unsupplemented Mueller-Hinton agar (MHA) or Mueller-Hinton broth (MHB). Alternative media and methods for some fastidious or uncommon organisms are described in Section 11 and M100[7] Tables 2E through 2L. Methods for testing anaerobic bacteria are outlined in CLSI document M11.[4] Methods for testing infrequently isolated or fastidious bacteria not included in M02[3] and M07 are found in CLSI document M45.[5]

This document, along with M100,[7] describes methods, QC, and interpretive criteria currently recommended for dilution susceptibility tests. When new problems are recognized or improvements in these criteria are developed, changes will be incorporated into future editions of this standard and also distributed in M100,[7] which provides annual informational supplements.

Related CLSI Reference Materials*

M02-A10 **Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Tenth Edition (2009).** This document contains the current Clinical and Laboratory Standards Institute-recommended methods for disk susceptibility testing, criteria for quality control testing, and updated tables for interpretive zone diameters.

M06-A2 **Protocols for Evaluating Dehydrated Mueller-Hinton Agar; Approved Standard—Second Edition (2006).** This document provides procedures for evaluating production lots of dehydrated Mueller-Hinton agar, and for developing and applying reference media.

M11-A7 **Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard—Seventh Edition (2007).** This document provides reference methods for the determination of minimal inhibitory concentrations (MICs) of anaerobic bacteria by agar dilution and broth microdilution.

M23-A3 **Development of *In Vitro* Susceptibility Testing Criteria and Quality Control Parameters; Approved Guideline—Third Edition (2008).** This document addresses the required and recommended data needed for the selection of appropriate interpretive criteria and quality control ranges for antimicrobial agents.

M29-A3 **Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Third Edition (2005).** Based on US regulations, this document provides guidance on the risk of transmission of infectious agents by aerosols, droplets, blood, and body substances in a laboratory setting; specific precautions for preventing the laboratory transmission of microbial infection from laboratory instruments and materials; and recommendations for the management of exposure to infectious agents.

M39-A2 **Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data; Approved Guideline—Second Edition (2005).** This document describes methods for the recording and analysis of antimicrobial susceptibility test data, consisting of cumulative and ongoing summaries of susceptibility patterns of clinically significant microorganisms.

M45-A **Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Approved Guideline (2006).** 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 documents M02, M07, 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.

M100-S19 **Performance Standards for Antimicrobial Susceptibility Testing; Nineteenth Informational Supplement (2009).** This document provides updated tables for the CLSI antimicrobial susceptibility testing standards M02-A10 and M07-A8.

* Proposed-level documents are being advanced through the Clinical and Laboratory Standards Institute consensus process; therefore, readers should refer to the most current editions.