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Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement

This document provides updated tables for the Clinical and Laboratory Standards Institute antimicrobial susceptibility testing standards M02-A10 and M07-A8.

An informational supplement for global application developed through the Clinical and Laboratory Standards Institute consensus process.

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

Clinical and Laboratory Standards Institute

Advancing Quality in Health Care Testing

The 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 health care 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 health care issues. Our process is based on the principle that consensus is an effective and cost-effective way to improve patient testing and health care services.

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

PUBLICATIONS

A 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 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 health care 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 health care community. It should be reviewed to assess the utility of the final document, to ensure attainment of consensus (ie, 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 addressed 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

Health care 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.

Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement

Abstract

The supplemental information presented in this document is intended for use with the antimicrobial susceptibility testing procedures published in the following Clinical and Laboratory Standards Institute (CLSI)–approved standards: M02-A10—*Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Tenth Edition*; and M07-A8—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Eighth Edition*. The standards contain information about both disk (M02) and dilution (M07) test procedures for aerobic bacteria.

Clinicians depend heavily on information from the clinical microbiology laboratory for treatment of their seriously ill patients. The clinical importance of antimicrobial susceptibility test results requires that these tests be performed under optimal conditions and that laboratories have the capability to provide results for the newest antimicrobial agents.

The tabular information presented here represents the most current information for drug selection, interpretation, and quality control using the procedures standardized in M02 and M07. Users should replace the tables published earlier with these new tables. (Changes in the tables since the most current edition appear in boldface type.)

Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement.* CLSI document M100-S20 (ISBN 1-56238-716-2). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2010.

The data in the interpretive tables in this supplement are valid only if the methodologies in M02-A10—*Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Tenth Edition*; and M07-A8—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Eighth Edition* are followed.

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The Clinical and Laboratory Standards Institute consensus process, which is the mechanism for moving a document through two or more levels of review by the health care community, is an ongoing process. Users should expect revised editions of any given document. Because rapid changes in technology may affect the procedures, methods, and protocols in a standard or guideline, users should replace outdated editions with the current editions of CLSI/NCCLS documents. Current editions are listed in the CLSI catalog and posted on our website at www.clsi.org. If your organization is not a member and would like to become one, and to request a copy of the catalog, contact us at: Telephone: +610.688.0100; Fax: +610.688.0700; E-mail: customerservice@clsi.org; Website: www.clsi.org.

Summary of Major Changes in This Document

This list includes the "major" changes in this document. Other minor or editorial changes have been made to the general formatting and to some of the table footnotes and comments. Boldface type is used to highlight the changes in each table.

Additions/Changes/Deletions

Major formatting changes:

The following table indicates renaming, renumbering, and relocating of several appendixes that were previously positioned at the end of M100-S19.

Previous Designation	New M100-S20 Designation/Location
• Appendix A (Screening and Confirmatory Tests for ESBLs)	• Supplemental Table 2A-S1/end of Table 2A
• Appendix G (Screening and Confirmatory Tests for Carbapenemases)	• Supplemental Table 2A-S2/end of Table 2A
• Appendix B (Screening Tests for *Staphylococcus aureus* Group)	• Supplemental Table 2C-S3/end of Table 2C
• Appendix C (Screening Tests for Coagulase-Negative Staphylococci)	• Supplemental Table 2C-S4/end of Table 2C
• Appendix D (Screening Tests for Enterococci)	• Supplemental Table 2D-S5/end of Table 2D
• Appendix E (Suggestions for Verification of AST Results)	• Appendix A/end of M100-S20 before Glossary
• Appendix F (QC Strains for AST)	• Appendix B/end of M100-S20 before Glossary

The following are additions or changes unless otherwise noted as a "***deletion.***"

Introduction to Tables 1 and 2

Revised the definition of nonsusceptible (p. 22)

Added information on using cephalothin breakpoints ONLY to predict susceptibility to other cephems (p. 30)

Added new Section VII describing Screening Tests, to include a summary of the screening tests, their limitations, and any tests needed to confirm results of the screening test (p. 26)

Tables 1 and 1A – Drugs Recommended for Testing and Reporting

Added cephamycins to the list of antimicrobial agents that should not be reported routinely for bacteria isolated from cerebrospinal fluid (CSF) located in the Warning box following Tables 1 and 1A (pp. 30 and 35)

Enterobacteriaceae:
Changed Test Report Group for cephalothin from A to U (pp. 28)

Acinetobacter spp.

Deleted colistin and polymyxin B from Test Report Group C

Summary of Major Changes in This Document (Continued)

Tables 1 and 1A – Drugs Recommended for Testing and Reporting (Continued)

Staphylococcus spp.:
Added information if testing a penicillinase-stable penicillin, oxacillin is the preferred agent and results can be applied to the other penicillinase-stable penicillins, cloxacillin, dicloxacillin, flucloxacillin, methicillin, and nafcillin (p. 32)

Modified footnotes in Table 1 to correspond with modifications of comments in Tables 2 as follows:

Footnote a) (p. 30) – see Enterobacteriaceae Table 2A comment (11) (p. 42)
Footnote k) (p. 31) – see *Staphylococcus* spp. Table 2C comment (9) (p. 62)

Tables 2A through 2L – Interpretive Criteria (Breakpoints)

***Enterobacteriaceae* (Table 2A):**
Added new (revised) breakpoints for cefazolin, cefotaxime, ceftazidime, ceftizoxime, ceftriaxone, and aztreonam. Also added dosage regimens on which the new breakpoints are based (pp. 42 and 43)

Added suggestion that when using the new breakpoints, routine ESBL testing is no longer necessary before reporting cephalosporin, penicillin, or aztreonam results (pp. 41 and 46)

Deleted former cefazolin disk diffusion breakpoints and noted that disk diffusion breakpoints to correlate with the new cefazolin MIC breakpoints have not yet been established (p. 42)

Added information indicating that results from testing cephalothin should be used ONLY to predict results for select oral cephems. Changed Test/Report Group for cephalothin from A to U (p. 42)

***Pseudomonas aeruginosa* (Table 2B-1):**
Deleted recommendation to add a report comment suggesting the addition of a second antimicrobial agent (eg, fluoroquinolone, aminoglycoside) for *P. aeruginosa* infections

***Staphylococcus* spp. (Table 2C):**
Clarified reporting results for β-lactam agents other than the cephalosporins with anti–methicillin-resistant *Staphylococcus aureus* (MRSA) activity for oxacillin-resistant *S. aureus* and coagulase-negative staphylococci (p. 60)

As related to vancomycin MIC results, modified recommendations for sending isolates of *S. aureus* and coagulase-negative *Staphylococcus* to a reference laboratory for confirmation (pp. 65 and 66)

Expanded definition of MRSA strains (p. 60)

Expanded discussion of limitations of β-lactamase testing for staphylococcal isolates that test susceptible to penicillin but might have the ability to produce β-lactamase (p. 62)

Clarified recommendations for reporting results when cefoxitin and oxacillin are tested against *S. aureus* or *S. lugdunensis* and either tests resistant (p. 62)

Added disk diffusion and MIC "resistant" interpretive criteria for linezolid (p. 68)

Further described cross-susceptibility of penicillinase-stable penicillins (p. 62)

Summary of Major Changes in This Document (Continued)

***Staphylococcus* spp. (Table 2C) (Continued):**

Reemphasized suggestions for performing additional testing of non–*S. epidermidis* coagulase-negative staphylococcal strains for which the oxacillin MIC is 0.5–2 µg/mL (p. 63)

Reemphasized recommendation that oxacillin would be the preferred agent to test if a penicillinase-stable penicillin is tested. (p. 62)

***Enterococcus* spp. (Table 2D):**
Modified recommendations for performing β-lactamase testing (p. 77)

***Streptococcus* spp. β-hemolytic Group (Table 2H-1):**
Modified the comment regarding cross susceptibility of penicillin and other β-lactams (p. 93)

***Streptococcus* spp. Viridans Group (Table 2H-2):**
Deleted suggestion that isolates that test susceptible to penicillin can be considered susceptible to other β-lactams

Clarified the organisms covered by Table 2H-2 recommendations (p. 96)

***Neisseria meningitidis* (Table 2J):**
Added an "or" between cefotaxime and ceftriaxone (p. 101)

Tables 3 and 4 – Quality Control

Disk Diffusion QC Ranges Changes/Additions (Table 3): (pp. 108–109)

Doripenem – *Escherichia coli* ATCC® 25922
Pseudomonas aeruginosa ATCC® 27853

Razupenem – *Escherichia coli* ATCC® 25922

Ulifloxacin (prulifloxacin) – *Escherichia coli* ATCC® 25922
Staphylococcus aureus ATCC® 25923
Pseudomonas aeruginosa ATCC® 27853

Disk Diffusion QC Ranges Changes/Additions (Table 3A): (p. 110)

Razupenem – *Streptococcus pneumoniae* ATCC® 49619
Haemophilus influenzae ATCC® 49247

MIC QC Ranges Changes/Additions (Table 4): (pp. 116–117)

Besifloxacin – *Staphylococcus aureus* ATCC® 29213
Enterococcus faecalis ATCC® 29212
Escherichia coli ATCC® 25922
Pseudomonas aeruginosa ATCC® 27853

Colistin – *Escherichia coli* ATCC® 25922
Pseudomonas aeruginosa ATCC® 27853

Daptomycin – *Staphylococcus aureus* ATCC® 29213

Summary of Major Changes in This Document (Continued)

MIC QC Ranges Changes/Additions (Table 4) (Continued):

Fidaxomicin – *Staphylococcus aureus* ATCC® 29213
Enterococcus faecalis ATCC® 29212

Polymyxin B – *Pseudomonas aeruginosa* ATCC® 27853

Razupenem – *Escherichia coli* ATCC® 25922
Staphylococcus aureus ATCC® 29213
Pseudomonas aeruginosa ATCC® 27853
Enterococcus faecalis ATCC® 29212

Teicoplanin – *Enterococcus faecalis* ATCC® 29212

Ulifloxacin (prulifloxacin) – *Escherichia coli* ATCC® 25922
Pseudomonas aeruginosa ATCC® 27853

MIC QC Ranges Changes/Additions (Table 4A): (pp. 118–119)

Besifloxacin – *Haemophilus influenzae* ATCC® 49247
Streptococcus pneumoniae ATCC® 49619

Linezolid – *Streptococcus pneumoniae* ATCC® 49619

Razupenem – *Haemophilus influenzae* ATCC® 49766
Streptococcus pneumoniae ATCC® 49619

Tetracycline – *Streptococcus pneumoniae* ATCC® 49619

Solvents and Diluents for Preparation of Stock Solutions of Antimicrobial Agents Changes/Additions (Table 5): (pp. 128–129)
Added: Besifloxacin
Fidaxomicin
Razupenem

Preparation of Solutions and Media Containing Combinations of Antimicrobial Agents (New) Table 5B
Explains methods for preparing stock solutions or media containing combinations of antimicrobial agents (pp. 132–133)

Cumulative Antimicrobial Susceptibility Report for *Bacteroides fragilis* Group Organisms (New) Appendix C
Provides guidance to laboratories on the percentage of certain anaerobes susceptible or resistant to commonly used antimicrobial agents. This table is also provided in the new M11-S1 Supplement (p. 143).

Glossaries I, II, and III:

Glossary I – added new antimicrobial subclass for ceftaroline and ceftobiprole (p. 144)

Glossaries I and II – added besifloxacin to fluoroquinolone subclass (pp. 145–146)
– added razupenem to carbapenem subclass (pp. 144 and 148)
– added ulifloxacin (prulifloxacin) to fluoroquinolone subclass (pp. 145 and 148)

Summary of CLSI Processes for Establishing Interpretive Criteria and Quality Control Ranges

The Clinical and Laboratory Standards Institute (CLSI) is an international, voluntary, nonprofit, interdisciplinary, standards-developing, and educational organization accredited by the American National Standards Institute (ANSI) that develops and promotes use of consensus-developed standards and guidelines within the health care community. These consensus standards and guidelines are developed to address critical areas of diagnostic testing and patient health care and are developed in an open and consensus seeking forum. CLSI is open to anyone or any organization that has an interest in diagnostic testing and patient care. Information about CLSI can be found at www.clsi.org.

The CLSI Subcommittee on Antimicrobial Susceptibility Testing (AST) reviews data from a variety of sources and studies (eg, *in vitro,* pharmacokinetics/pharmacodynamics, and clinical studies) to establish antimicrobial susceptibility test methods, interpretive criteria, and quality control (QC) parameters. The details of the data required to establish interpretive criteria, QC parameters, and how the data are presented for evaluation are described in CLSI document M23—*Development of* In Vitro *Susceptibility Testing Criteria and Quality Control Parameters.*

Over time, a microorganism's susceptibility to an antimicrobial agent may decrease, resulting in a lack of clinical efficacy and/or safety. In addition, microbiological methods and QC parameters may be refined to ensure more accurate and better performance of susceptibility test methods. Because of this, CLSI continually monitors and updates information in its documents. Although CLSI standards and guidelines are developed using the most current information and thinking available at the time, the field of science and medicine is ever changing; therefore, standards and guidelines should be used in conjunction with clinical judgment, current knowledge, and clinically relevant laboratory test results to guide patient treatment.

Additional information, updates, and changes in this document can be found in the meeting summary minutes of the Subcommittee on Antimicrobial Susceptibility Testing at www.clsi.org.

CLSI Reference Methods vs Commercial Methods and CLSI vs FDA Breakpoints (interpretive criteria)

It is important for users of M02-A10, M07-A8, and the M100 Informational Supplement to recognize that the standard methods described in CLSI documents are reference methods. These methods may be used for routine antimicrobial susceptibility testing of clinical isolates, for evaluation of commercial devices that will be used in clinical laboratories, or by drug or device manufacturers for testing of new agents or systems. Results generated by reference methods, such as those contained in CLSI documents, may be used by regulatory authorities to evaluate the performance of commercial susceptibility testing devices as part of the approval process. Clearance by a regulatory authority indicates that the commercial susceptibility testing device provides susceptibility results that are substantially equivalent to results generated using reference methods for the organisms and antimicrobial agents described in the device manufacturer's approved package insert.

CLSI breakpoints may differ from those approved by various regulatory authorities for many reasons, including the following: different databases, differences in interpretation of data, differences in doses used in different parts of the world, and public health policies. Differences also exist because CLSI proactively evaluates the need for changing breakpoints. The reasons why breakpoints may change and the manner in which CLSI evaluates data and determines breakpoints are outlined in CLSI document M23—*Development of* In Vitro *Susceptibility Testing Criteria and Quality Control Parameters*.

Following a decision by CLSI to change an existing breakpoint, regulatory authorities may also review data in order to determine how changing breakpoints may affect the safety and effectiveness of the antimicrobial agent for the approved indications. If the regulatory authority changes breakpoints, commercial device manufacturers may have to conduct a clinical laboratory trial, submit the data to the regulatory authority, and await review and approval. For these reasons, a delay of more than the suggested CLSI "tentative" period of one year may be required if an interpretive breakpoint change is to be implemented by a device manufacturer. In the United States, laboratories that use Food and Drug Administration (FDA)–approved susceptibility testing devices are allowed to use existing FDA interpretive breakpoints. Either FDA or CLSI susceptibility interpretive breakpoints are acceptable to clinical laboratory accrediting bodies. Policies in other countries may vary.

Following discussions with appropriate stakeholders such as infectious disease practitioners and the pharmacy department, as well as the Pharmacy and Therapeutics and Infection Control committees of the medical staff, newly approved or revised breakpoints may be implemented by clinical laboratories. CLSI disk diffusion test breakpoints may be implemented as soon as they are published in M100. If a device includes antimicrobial test concentrations sufficient to allow interpretation of susceptibility and resistance to an agent using the CLSI breakpoints, a laboratory could, after appropriate validation, choose to interpret and report results using CLSI breakpoints.

Subcommittee on Antimicrobial Susceptibility Testing Mission Statement

The Subcommittee on Antimicrobial Susceptibility Testing is composed of representatives from the professions, government, and industry, including microbiology laboratories, government agencies, health care 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 Subcommittee on Antimicrobial Susceptibility Testing is to

- Develop standard reference methods for antimicrobial susceptibility tests.
- Provide QC 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 detection of emerging resistance mechanisms through development of new or revised methods, interpretive criteria, and QC parameters.
- Educate users through multimedia communication of standards and guidelines.
- Foster a dialog 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.

Introduction to Tables 1 and 2 for Use With M02-A10 (Disk Diffusion) and M07-A8 (MIC Testing)

On the following pages, you will find

1. Tables 1, 1A, and 1B—Suggested groupings of antimicrobial agents that should be considered for routine testing and reporting by clinical microbiology laboratories. These guidelines are based on drugs with clinical indications approved by the US Food and Drug Administration (FDA) in the United States. In other countries, placement of antimicrobial agents in Tables 1, 1A, and 1B should be based on available drugs approved for clinical use by relevant regulatory agencies.

2. For each organism group, an additional table (Tables 2A through 2L) contains
   a. Recommended testing conditions.
   b. Minimal quality control (QC) recommendations. (See also the text documents M02-A10, Section 15 and M07-A8, Section 17.)
   c. General comments for testing the organism group and specific comments for testing particular drug/organism combinations.
   d. Suggested agents that should be considered for routine testing and reporting by clinical microbiology laboratories, as specified in Tables 1, 1A, and 1B (test/report groups A, B, C, U).
   e. Additional drugs that have an approved indication for the respective organism group but would generally not warrant routine testing by a clinical microbiology laboratory in the United States (test/report group O for "other"; test/report group Inv. for "investigational" [not yet FDA approved]).
   f. Zone diameter breakpoints and minimal inhibitory concentration (MIC) interpretive standard criteria.

3. For some organism groups, a supplemental table summarizing screening tests that may be appropriate for use with isolates within the organism group.

I. Selecting Antimicrobial Agents for Testing and Reporting

A. Selection of the most appropriate antimicrobial agents to test and to report is a decision best made by each clinical laboratory in consultation with the infectious disease practitioners and the pharmacy, as well as the pharmacy and therapeutics and infection control committees of the medical staff. The recommendations here for each organism group comprise agents of proven efficacy that show acceptable *in vitro* test performance. Considerations in the assignment of agents to specific test/report groups include clinical efficacy, prevalence of resistance, minimizing emergence of resistance, cost, FDA clinical indications for use, and current consensus recommendations for first-choice and alternative drugs. Unexpected resistance should be reported (eg, resistance of *Enterobacteriaceae* to carbapenems). Tests of selected agents may be useful for infection control purposes.

B. The listing of drugs together in a single box designates clusters of agents for which interpretive results (susceptible, intermediate, or resistant) and clinical efficacy are similar. Within each box, an "or" between agents designates those agents for which cross resistance and cross susceptibility are nearly complete. This means combined major and very major errors are fewer than 3% and minor errors are fewer than 10%, based on a large population of bacteria tested. In addition, to qualify for an "or," at least 100 strains with resistance to the agents in question must be tested and a result of "resistant" must be obtained with all agents for at least 95% of the strains. "Or" is also used for comparable antimicrobial agents when tested against organisms for which "susceptible-only" interpretive criteria are provided (eg, cefotaxime or ceftriaxone with *Haemophilus*

M02-A10
Vol. 29 No. 1
Replaces M02-A9
Vol. 26 No. 1

January 2009

Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Tenth Edition

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.

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

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

Contents (Continued)

Contents (Continued)

Foreword

In this 2009 revision of CLSI document M02, several sections have been added or revised as outlined below 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 M02 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 M02 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 working group chairholders 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.

Summary of Major Changes in This Document (Continued)

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

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 7.1, Mueller-Hinton agar
Section 8.1, 0.5 McFarland standard
Section 10.1, *Haemophilus* Test Medium (HTM)
Section 10.2, GC agar
Sections 10.3 and 10.4, Mueller-Hinton agar supplemented with 5% sheep blood

Section 7.3.1, Source of Disks and Information About Disks
Added recommendations to ensure appropriate disks are used.

Section 9.3, Reading Plates and Interpreting Results
Added guidance for reading zone diameters (eg, cefoxitin or linezolid when tested against *Staphylococcus* spp.).

Section 10.3, *Neisseria meningitidis*
Added cautionary statement for performing susceptibility testing in a biological safety cabinet.

Section 11.1.3.1, Methods for Detection of Reduced Susceptibility to Vancomycin
Added table summarizing the various methods to detect levels of vancomycin susceptibility in *S. aureus*.

Section 11.1.3.3, Heteroresistant Vancomycin-Intermediate *Staphylococcus aureus* (hVISA)
Added discussion of hVISA.

Section 11.1.5, Linezolid Resistance
Added recommendations for reading linezolid zones using transmitted light.

Section 11.1.6, Mupirocin Resistance
Added method for detecting and reporting high-level mupirocin resistance (ie, MICs ≥ 512 µg/mL) in *S. aureus*.

Section 11.3, 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 15.2, Quality Control Responsibilities
Added new section outlining the quality control responsibilities of both manufacturers and users.

Section 15.3, Selection of Quality 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 15.7.1, Daily Testing
Clarified consecutive results as consecutive test days.

Summary of Major Changes in This Document (Continued)

Section 15.8.1, Out-of-Control Result Due to Identifiable Error
Expanded on the possible causes for out-of-control results and strategy for corrective action.

Section 15.8.2, Out-of-Control Result With No Error Identified
Expanded on the possible causes for out-of-control results and strategy for corrective action.

Section 15.11, Other Control Procedures
Added section outlining inoculum control and end-point interpretation control.

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

Appendix C, Conditions for Disk Diffusion 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
Added new appendix providing quality control organism characteristics.

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

Summary of CLSI Processes for Establishing Interpretive Criteria and QC Ranges

The Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) is an international, voluntary, nonprofit, interdisciplinary, standards-developing, and educational organization accredited by the American National Standards Institute (ANSI), that develops and promotes use of consensus developed standards and guidelines within the health care community. These consensus standards and guidelines are developed to address critical areas of diagnostic testing and patient health care and are developed in an open and consensus seeking forum. CLSI is open to anyone, or any organization that has an interest in diagnostic testing and patient care. Information about CLSI is found at www.clsi.org.

The CLSI Subcommittee on Antimicrobial Susceptibility Testing reviews data from a variety of sources and studies (eg, *in vitro*, pharmacokinetics/pharmacodynamics, and clinical studies) to establish antimicrobial susceptibility test methods, interpretive criteria, and quality control (QC) parameters. The details of the data required to establish interpretive criteria, QC parameters, and how the data are to be presented for evaluation are described in CLSI document M23.[1]

Over time, a microorganism's susceptibility to an antimicrobial agent may decrease, resulting in a lack of clinical efficacy and/or safety. In addition, microbiological methods and QC parameters may be refined to ensure more accurate and better performance of susceptibility test methods. Because of this, CLSI continually monitors and updates information in its documents. While CLSI standards and guidelines are developed using the most current information and thinking available at the time, the field of science and medicine is ever changing; therefore, standards and guidelines should be used in conjunction with clinical judgment, current knowledge, and clinically relevant laboratory test results to guide patient treatment.

Additional information, updates, and changes in this document are found in the meeting summary minutes of the Subcommittee on Antimicrobial Susceptibility Testing at www.clsi.org.

CLSI Reference Methods vs Commercial Methods and CLSI vs FDA Breakpoints (interpretive criteria)

It is important for users of M02-A10 and M07-A8 to recognize that commercial susceptibility testing devices are not addressed in these standards. 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 US Food and Drug Administration (FDA) 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.

CLSI breakpoints may differ from those approved by various regulatory authorities for many reasons, including the following: different databases, differences in interpretation of data, differences in doses utilized in different parts of the world, and public health policies. Differences also exist because CLSI proactively evaluates the need for changing breakpoints. The reasons why breakpoints may change and the manner in which CLSI evaluates data and determines breakpoints are outlined in CLSI document M23—*Development of* In Vitro *Susceptibility Testing Criteria and Quality Control Parameters*.

Following a decision by CLSI to change an existing breakpoint, regulatory authorities may also review data in order to determine how changing breakpoints may affect the safety and effectiveness of the antimicrobial agent for the approved indications. If the regulatory authority changes breakpoints, commercial device manufacturers may have to conduct a clinical laboratory trial, submit the data to the regulatory authority, and await review and approval. For these reasons, a delay of more than the suggested CLSI "tentative" period of one year may be required if an interpretive breakpoint change is to be implemented by a device manufacturer. In the United States, laboratories that use Food and Drug Administration (FDA)-approved susceptibility testing devices are allowed to utilize existing FDA interpretive breakpoints. Either FDA or CLSI susceptibility interpretive breakpoints are acceptable to clinical laboratory accrediting bodies. Policies in other countries may vary.

Following discussions with appropriate stakeholders such as infectious disease practitioners and the pharmacy department, as well as the Pharmacy and Therapeutics and Infection Control committees of the medical staff, newly approved or revised breakpoints may be implemented by clinical laboratories. CLSI disk diffusion test breakpoints may be implemented as soon as they are published in M100. If a device includes antimicrobial test concentrations sufficient to allow interpretation of susceptibility to an agent using the CLSI breakpoints, a laboratory could, after appropriate validation, choose to interpret and report results using CLSI breakpoints.

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Key Words

Agar diffusion, antibiotic, antimicrobial agents, disk diffusion, Kirby-Bauer method, susceptibility testing

Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Tenth Edition

1 Scope

This document describes the standard agar disk diffusion techniques used to determine the *in vitro* susceptibility of bacteria that grow aerobically. The document addresses preparation of agar plates, testing conditions (including inoculum preparation and standardization, incubation time, and incubation temperature), interpretation of results, quality control (QC) procedures, and limitations of disk diffusion 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 dilution methods are found in CLSI document M07[2]; standards for testing the *in vitro* susceptibility of bacteria that grow anaerobically are found in CLSI document M11.[3] Guidelines for standardized susceptibility testing of infrequently isolated or fastidious bacteria that are not included in CLSI documents M02, M07,[2] or M11[3] are available in CLSI document M45.[4]

2 Introduction

A variety of laboratory methods can be used to measure the *in vitro* susceptibility of bacteria to antimicrobial agents. In many clinical microbiology laboratories, an agar disk diffusion method is used routinely for testing common, rapidly growing, and certain fastidious bacterial pathogens. This document describes the performance, applications, and limitations of the standardized disk diffusion test method. Recommendations of the International Collaborative Study (ICS)[5] and regulations[6,7] proposed by the US Food and Drug Administration (FDA) have been reviewed, and appropriate sections have been incorporated into this standard. Other susceptibility testing methods exist that provide essentially equivalent results to the CLSI methods described herein. The FDA is responsible for the approval of commercial devices used in the United States. CLSI does not approve or endorse commercial products or devices.

Disk diffusion tests based solely on the presence or absence of a zone of inhibition without regard to the size of the zone are not acceptable for determining antimicrobial susceptibility. Reliable results can only be obtained with disk diffusion tests that use the principle of standardized methodology and zone diameter measurements correlated with minimal inhibitory concentrations (MICs) with strains known to be susceptible or resistant to various antimicrobial agents.

The methods described herein must be followed explicitly to obtain reproducible results. The standardized method currently recommended by the CLSI Subcommittee on Antimicrobial Susceptibility Testing is based on the method originally described by Bauer et al.[8] This is the most thoroughly described disk diffusion method for which interpretive standards have been developed and supported by laboratory and clinical data.

This document describes methods, QC, and interpretive criteria recommended presently for disk diffusion 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 annual informational supplements (M100).[9]

3 Standard Precautions

Because it is often impossible to know what isolates or specimens might be infectious, all patient and laboratory specimens are treated as infectious and handled according to "standard precautions." Standard precautions are guidelines that combine the major features of "universal precautions and body substance

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Replaces M07-A7
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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.

Contents (Continued)

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Foreword

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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.

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Summary of Major Changes in This Document (Continued)

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

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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.

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Section 16.7.1, Daily Testing
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Section 16.9.2, Out-of-Control Result With No Error Identified
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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
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- 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 detection of emerging resistance mechanisms through development of new or revised methods, interpretive criteria, and quality control parameters.
- Educate users through multimedia communication of standards and guidelines.
- 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.

Key Words

Agar dilution, antimicrobial susceptibility, broth dilution, macrodilution, microdilution, minimal inhibitory concentration (MIC)

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.