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M07

Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically

This standard covers reference methods for determining minimal inhibitory concentrations 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

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Abstract

Antimicrobial 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.

Various laboratory methods can be used to measure the *in vitro* susceptibility of bacteria to antimicrobial agents. Clinical and Laboratory Standards Institute standard M07—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically* 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[2] tables) used with this standard represents the most current information for drug selection, interpretation, and quality control using the procedures standardized in M07.

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Contents

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Foreword

The most current edition of CLSI document M100,[2] an annually published volume of tables, is made available with this standard to ensure users are aware of the latest recommendations related to the methods described in M07 and CLSI document M02.[3]

Many other editorial and procedural changes in this edition of M07 resulted from Subcommittee on Antimicrobial Susceptibility Testing meetings held since 2015. Specific changes to the tables are summarized at the beginning of M100.[2] The most important changes in M07 are summarized below.

Overview of Changes

This standard replaces the previous edition of the approved standard, M07-A10, published in 2015. Several changes were made in this edition, including:

- **General:**
  - Harmonized language and information on drug selection and QC with CLSI document M02[3]
  - To harmonize with the International Organization for Standardization, the terms for the methods for inoculum preparation have been changed. "Growth method" has been changed to "broth culture method," and "direct colony suspension method" has been changed to "colony suspension method" throughout the document
- **Subchapter 1.4.1, Definitions:**
  - Clarified definitions for breakpoint, interpretive category, susceptible, susceptible-dose dependent, intermediate, resistant, nonsusceptible, and quality control
  - Added definitions for minimal inhibitory concentration, routine test, supplemental test, surrogate agent test, CarbaNP test, and modified carbapenem inactivation method
- **Subchapter 1.4.2, Abbreviations and Acronyms:**
  - Deleted abbreviations for β-lactamase types
- **Subchapter 2.3, Antimicrobial Agent Classes:**
  - Clarified and updated antimicrobial agent classes
- **Subchapter 2.3.2.2, Folate Pathway Antagonists:**
  - Revised nomenclature from "folate pathway inhibitor" to "folate pathway antagonist"
- **Subchapter 3.9, Determining Broth Macro- or Microdilution End Points:**
  - Added photographs of growth control examples and for interpreting skipped wells
- **Subchapter 3.11, Table 1. Testing Considerations for Fastidious Organisms:**
  - Clarified source plate incubation times and inoculum broth for some fastidious organisms
- **Subchapter 3.12, Special Considerations for Detecting Resistance:**
  - Reorganized and streamlined
  - Moved Subchapters 3.12.4 (Inducible Clindamycin Resistance) and 3.12.6 (β-Lactamase Tests) to create a new subchapter, 3.13 (Supplemental [Not Routine] Tests)

- **Subchapter 3.12.1, Staphylococci:**
  - Added information for *Staphylococcus pseudintermedius* and *Staphylococcus schleiferi*
  - Reorganized and clarified information for staphylococci

- **Subchapter 3.12.4, Gram-Negative Bacilli:**
  - Expanded and clarified information on β-lactamases
  - Added footnote to Table 4, Enzyme Classifications for β-Lactamases, to clarify the difference between cephalosporin subclasses and generations
  - Updated nomenclature for *Enterobacter aerogenes* to *Klebsiella* (formerly *Enterobacter*) *aerogenes*[4]

- **Subchapter 3.13.1, Inducible Clindamycin Resistance:**
  - Consolidated information from former Subchapter 3.13.1.8

- **Subchapter 4.3, Selecting Strains for Quality Control:**
  - Clarified the example in the third paragraph

- **Appendixes:**
  - Reorganized to reflect the order in which they are referenced in the main text, as follows:
    - **Appendix A. Preparation of Supplements, Media, and Reagents** (formerly Appendix B)
    - **Appendix B. Conditions for Dilution Antimicrobial Susceptibility Tests** (formerly Appendix C)
    - **Appendix C. Quality Control Strain Maintenance** (formerly Appendix E)
    - **Appendix D. Quality Control Protocol Flow Charts** (formerly Appendix A)
  - Deleted **Quality Control Strains for Antimicrobial Susceptibility Tests** (formerly Appendix D) (see M100[2] Appendix C)

- **Appendix A. Preparation of Supplements, Media, and Reagents:**
  - Reorganized procedures into step-action tables

- **Appendix C. Quality Control Strain Maintenance:**
  - Clarified maintenance and subculture of QC strains

- **Appendix D. Quality Control Protocol Flow Charts:**
  - Recreated QC flow charts in black-and-white format for easier viewing
  - Revised Appendixes D1 and D2 flow charts

Summary of CLSI Processes for Establishing Breakpoints and Quality Control Ranges

The Clinical and Laboratory Standards Institute (CLSI) is an international, voluntary, not-for-profit, interdisciplinary, standards-developing, and educational organization accredited by the American National Standards Institute that develops and promotes the use of consensus-developed standards and guidelines within the health care community. These consensus standards and guidelines are developed in an open and consensus-seeking forum to cover critical areas of diagnostic testing and patient health care. 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, breakpoints, and QC parameters. The details of the data necessary to establish breakpoints, QC parameters, and how the data are presented for evaluation are described in CLSI document M23.[5]

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 these types of changes, CLSI continually monitors and updates information in its documents. Although CLSI standards and guidelines are developed using the most current information available at the time, the field of science and medicine is always 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 standard 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 US Food and Drug Administration Breakpoints

It is important for users of M02,[3] M07, and the M100[2] 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 patient isolates, for evaluation of commercial devices that will be used in medical 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 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 use of different databases, differences in data interpretation, 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.[5]

Following a decision by CLSI to change an existing breakpoint, regulatory authorities may also review data 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 trial, submit the data to the regulatory authority, and await review and approval. For these reasons, a delay of one or more years may be needed if a breakpoint and interpretive category change is to be implemented by a device manufacturer. In the United States, it is acceptable for laboratories that use US Food and Drug Administration (FDA)–cleared susceptibility testing devices to use existing FDA breakpoints. Either FDA or CLSI susceptibility breakpoints are acceptable to laboratory accrediting organizations in the United States. Policies in other countries may vary. Each laboratory should check with the manufacturer of its antimicrobial susceptibility test system for additional information on the breakpoints and interpretive categories used in its system's software.

Following discussions with appropriate stakeholders (eg, infectious diseases and pharmacy practitioners, the pharmacy and therapeutics and infection control committees of the medical staff, and antimicrobial stewardship teams), newly approved or revised breakpoints may be implemented by laboratories. Following verification, CLSI broth dilution and agar dilution test breakpoints may be implemented as soon as they are published in M100.[2] 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 choose to, after appropriate verification, 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 quality control parameters for standard test methods.
- Establish breakpoints for the results of standard antimicrobial susceptibility tests and provide epidemiological cutoff values when breakpoints are not available.
- 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, breakpoints, 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.

NOTE: The content of this standard is supported by the CLSI consensus process and does not necessarily reflect the views of any single individual or organization.

Key Words

Agar dilution, antimicrobial susceptibility, broth dilution, broth macrodilution, broth microdilution, minimal inhibitory concentration

Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically

Chapter 1: Introduction

This chapter includes:

- Standard's scope and applicable exclusions
- Background information pertinent to the standard's content
- Standard precautions information
- Terms and definitions used in the standard
- Abbreviations and acronyms used in the standard

1.1 Scope

This standard describes standard broth (macrodilution and microdilution) and agar dilution methods for determining *in vitro* susceptibility to antimicrobial agents for bacteria that grow aerobically and includes:

- Broth and agar dilution test preparation
- Testing conditions, including inoculum preparation and standardization, incubation time, and incubation temperature
- Reporting minimal inhibitory concentration (MIC) results
- QC procedures
- Dilution test method limitations

To assist the medical laboratory, suggestions are provided for selecting antimicrobial agents for routine testing and reporting.

Standards for testing the *in vitro* antimicrobial susceptibility of bacteria that grow aerobically using the antimicrobial disk testing method are found in CLSI document M02.[3] Standards for testing the *in vitro* antimicrobial susceptibility of bacteria that grow anaerobically are found in CLSI document M11.[6] Guidelines for standardized antimicrobial susceptibility testing (AST) of infrequently isolated or fastidious bacteria that are not included in CLSI documents M02,[3] M07, or M11[6] are available in CLSI document M45.[7] The AST methods provided in this standard can be used in laboratories around the world including but not limited to:

- Medical laboratories
- Public health laboratories
- Research laboratories
- Food laboratories
- Environmental laboratories

1.2 Background

Either broth or agar dilution methods may be used to quantitatively measure the *in vitro* activity of an antimicrobial agent against a given bacterial isolate. To perform the tests, plates or a series of tubes are prepared with an agar or broth medium to which various concentrations of the antimicrobial agents are added. The plates or tubes are then inoculated with a standardized suspension of the test organism. After incubating for the appropriate time interval, the tests are read, the MIC is determined, and the results are analyzed using approved breakpoints. The final result is significantly influenced by methodology, which must be carefully controlled if reproducible results (intra- and interlaboratory) are to be achieved.

This standard describes reference broth dilution (macrodilution and microdilution) and agar dilution methods. The basic components of these methods are largely derived from information contained in published recommendations.[8] Although these methods are standard reference methods, some are sufficiently practical for routine use in medical or public health laboratories.

Commercial systems based primarily or in part on some of these methods are available and may provide results essentially equivalent to the CLSI methods described. CLSI does not approve or endorse commercial products or devices.

The methods described in this standard 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 Subchapter 3.11 and M100[2] Tables 2E through 2I. Methods for testing anaerobic bacteria are provided in CLSI document M11[6] and in M100[2] Table 2J. Methods for testing infrequently isolated or fastidious bacteria not included in CLSI documents M02[3] and M07 are found in CLSI document M45.[7]

This standard, along with M100,[2] describes methods, QC, breakpoints, and interpretive categories 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 M100.[2]

1.3 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 isolation" practices. Standard precautions cover the transmission of all known infectious agents and thus are more comprehensive than universal precautions, which are intended to apply only to transmission of bloodborne pathogens. Published guidelines are available that discuss the daily operations of diagnostic medicine in humans and animals while encouraging a culture of safety in the laboratory.[9] For specific precautions for preventing the laboratory transmission of all known infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all known infectious diseases, refer to CLSI document M29.[10]

1.4 Terminology

1.4.1 Definitions

breakpoint – minimal inhibitory concentration (MIC) or zone diameter value used to categorize an organism as susceptible, susceptible-dose dependent, intermediate, resistant, or nonsusceptible; **NOTE 1:** MIC or zone diameter values generated by a susceptibility test can be interpreted based upon established breakpoints; **NOTE 2:** See **interpretive category.**

