CLSI M07™

Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically

CLSI M07 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.
Abstract

Clinical and Laboratory Standards Institute M07—Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically offers guidance for antimicrobial susceptibility testing that 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 M07—Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically describes standard broth dilution (macrodilution and microdilution [the microdilution method described in CLSI M07 is the same methodology outlined in ISO 20776-1]) 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 (CLSI M100–S tables) used with this standard represents the most current information for drug selection, interpretation, and QC using the procedures standardized in CLSI M07.

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Foreword

The most current edition of CLSI M100, 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 CLSI M02, M07, and M11. Many other editorial and procedural changes in this edition of CLSI M07 resulted from Subcommittee on Antimicrobial Susceptibility Testing meetings held since 2018. Specific changes to the tables are summarized at the beginning of CLSI M100. The most important changes in CLSI M07 are summarized below.

Overview of Changes

This standard replaces CLSI M07-Ed11, published in 2018. Several changes were made in this edition, including:

- **General:**
  - Revised information for testing and reporting to clarify relevant stakeholders
  - Revised nomenclature from “coagulase-negative staphylococci” (CoNS) to “staphylococci other than Staphylococcus aureus” (SOSA)
  - Revised nomenclature from “groups” to “tiers”
  - Revised nomenclature from “microdilution trays” to “microdilution panels”

- **Subchapter 1.4.1, Definitions:**
  - Added definitions for antimicrobial susceptibility testing, EDTA-modified carbapenem inactivation method, heteroresistance, inducible clindamycin resistance, and reference method
  - Deleted definition for D-zone test

- **Subchapter 1.4.2, Abbreviations and Acronyms:**
  - Added abbreviations for Mueller-Hinton fastidious and staphylococci other than S. aureus
  - Deleted abbreviations for coagulase-negative staphylococci and Mueller-Hinton broth

- **Subchapter 2.1, Selecting Antimicrobial Agents for Routine Testing and Reporting:**
  - Clarified and updated information on selecting antimicrobial agents for routine reporting

- **Subchapter 2.2, Equivalent Agents:**
  - Replaced former Subchapter 2.2, Routine Reports, with Subchapter 2.2, Equivalent Agents, with information on antimicrobial agents listed in CLSI M100 Tables 1

- **Subchapter 2.3, Suggested Guidelines for Routine and Selective Testing and Reporting:**
  - Revised suggested guidelines for routine and selective testing and reporting
  - Added new Table 2, Antimicrobial Agent Test and Report Tiers and Additional Considerations for Agents Listed in CLSI M100 Tables 1
  - Added new Table 3, Antimicrobial Agent Test and Report Designations and Additional Considerations for Agents Not Listed in CLSI M100 Tables 1

- **Subchapter 2.4, Antimicrobial Classes:**
  - Added new Table 4, Antimicrobial Agent Class Characteristics
• Subchapter 3.1.3, Preparing Stock Solutions:
  – Added information stating that if the addition of a surfactant does not result in enhanced or reduced activity of the antimicrobial agent, a small amount of surfactant may be added to the stock solution

• Subchapter 3.1.4, Number of Concentrations Tested:
  – Added information about having QC organisms that are on scale and cover most minimal inhibitory concentration ranges included in the concentration being tested

• Subchapter 3.2.2, Colony Suspension Methods for Inoculum Preparation:
  – Revised colony suspension method for inoculum preparation

• Subchapter 3.3, Agar Dilution Procedure:
  – Added information explaining that data have not been reviewed for all recent antimicrobial agents
  – Added information stating that agar dilution is the only approved method for testing fosfomycin

• Subchapter 3.5.1, Cation-Adjusted Mueller-Hinton Broth:
  – Added iron-depleted cation-adjusted Mueller-Hinton broth as the recommended medium for antimicrobial susceptibility testing of commonly isolated, rapidly growing, aerobic or facultative organisms when cefiderocol is tested

• Subchapter 3.5.2, Broth Media for Testing Fastidious Organisms:
  – Added Mueller-Hinton fastidious broth to list of media that can be used for testing fastidious organisms

• Subchapter 3.6.1, Preparing and Storing Diluted Antimicrobial Agents:
  – Deleted sentence stating that tubes from broth macrodilution can be saved for later use only if they have been frozen

• Subchapter 3.6.2, Inoculum Preparation, Inoculation, and Incubation:
  – Added minimum final volume for broth macrodilution

• Subchapter 3.7.1, Preparing and Storing Diluted Antimicrobial Agents:
  – Added information stating that an automated instrument or liquid handling device can be used
  – Added information stating that the dilution scheme and volumes should be delivered according to the dispensing system

• Subchapter 3.7.2, Inoculum Preparation, Inoculation, and Incubation:
  – Added examples for determining appropriate colony-forming units for each microdilution well when the microdilution method is used

• Subchapter 3.8.1, Performing Colony Counts From a 0.5 McFarland Suspension:
  – Added a step-action table for performing colony counts from a 0.5 McFarland suspension

• Subchapter 3.8.2, Performing Colony Counts From a Microdilution Well or Macrodilution Tube:
  – Added new subchapter

• Subchapter 3.9, Determining Broth Macro- or Microdilution End Points:
  – Added information on interpreting results
  – Revised information about trailing growth and reading minimum inhibitory concentrations
• **Subchapter 3.11, Special Considerations for Fastidious Organisms:**
  - Added information on Mueller-Hinton fastidious broth for testing *Haemophilus influenzae*

• **Subchapter 3.12, Special Considerations for Detecting Resistance:**
  - Added information about meC isolates to Subchapter 3.12.1.3.2, Reporting Oxacillin Resistance
  - Revised information on vancomycin-resistant *S. aureus* and vancomycin-intermediate *S. aureus* in Subchapter 3.12.1.4, Vancomycin Resistance in *S. aureus*
  - Revised information on vancomycin agar screen for *S. aureus* in Subchapter 3.12.1.4.2, Vancomycin Agar Screen for *S. aureus*
  - Revised information on AmpC enzymes in Subchapter 3.12.4.2, AmpC Enzymes
  - Revised information on carbapenemases in Subchapter 3.12.4.3, Carbapenemases (Carbapenem-Resistant Gram-Negative Bacilli)
  - Deleted former Subchapter 3.12.1.6, Linezolid Resistance
  - Renumbered former Table 2, Methods for Detecting Oxacillin Resistance in Staphylococci, as Table 6, Methods or Targets for Detection of Methicillin (Oxacillin)-Resistant *Staphylococcus* spp., and revised incubation times and methods
  - Renumbered former Table 3, Procedural Recommendations for Detecting Oxacillin Resistance in Staphylococci, as Table 7, Procedural Recommendations for Detection of Methicillin (Oxacillin)-Resistant *Staphylococcus* spp.

• **Subchapter 3.14.3, Development of Resistance and Testing Repeat Isolates:**
  - Revised information on development of resistance

• **Subchapter 4.3, Selecting Strains for Quality Control:**
  - Added recommendation for using additional or supplemental QC strains with end points covering as many drug concentrations on the panel as possible

• **Appendix A, Preparation of Supplements, Media, and Reagents:**
  - Added new section A3.5, Mueller-Hinton Fastidious Broth

• **Appendix B, Conditions for Dilution Antimicrobial Susceptibility Tests:**
  - Table B1, Conditions for Dilution Antimicrobial Susceptibility Tests for Nonfastidious Organisms
    - Added iron-depleted cation-adjusted Mueller-Hinton broth (ceferodol) as an acceptable medium for testing some nonfastidious organisms
    - Added water as an acceptable medium for preparing 0.5 McFarland inoculum for testing nonfastidious organisms
  - Table B2, Conditions for Dilution Antimicrobial Susceptibility Tests for Fastidious Organisms
    - Added Mueller-Hinton fastidious agar as an acceptable medium for testing for *H. influenzae* and *Streptococcus pneumoniae*
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 can be 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 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 CLSI Subcommittee on Antimicrobial Susceptibility Testing at https://clsi.org/meetings/ast-file-resources/.
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, 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 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 breakpoints and interpretive categories 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 QC 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

<table>
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<tr>
<th>agar dilution</th>
<th>broth dilution</th>
<th>broth microdilution</th>
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<td>antimicrobial susceptibility</td>
<td>broth macrodilution</td>
<td>minimal inhibitory concentration</td>
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Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically

Introduction

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
- Results interpretation
- QC procedures
- Dilution test method limitations

To assist the medical laboratory, recommendations 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 M02.3 Standards for testing the in vitro antimicrobial susceptibility of bacteria that grow anaerobically are found in CLSI M11.4 Guidelines for standardized antimicrobial susceptibility testing (AST) of infrequently isolated or fastidious bacteria that are not included in CLSI M02, M07, or M11 are available in CLSI M45.6 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, a series of tubes or microtiter wells with a broth (for broth dilution) or agar medium (for agar dilution) are prepared to which various concentrations of the antimicrobial agents are added. The tubes or microtiter wells (for broth dilution) or plates (for agar dilution) are then inoculated with a standardized suspension of the test organism. After incubating for the appropriate time interval, the tests are read, the minimal inhibitory concentration (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.
# Broth and Agar Dilution Antimicrobial Susceptibility Testing Process

Figure 1 provides an overview of the disk diffusion AST process. Detailed information for each step is provided in each designated chapter or subchapter.

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**Figure 1. Broth and Agar Dilution AST Process**

Abbreviations: AST, antimicrobial susceptibility testing; QC, quality control.

1. Three basic symbols are used in this process flow chart: oval (signifies the beginning or end of a process), arrow (connects process activities), box (designates process activities).
2. See CLSI M100.²
3. See CLSI M07 Minimal Inhibitory Concentration Reading Guide.³