CLINICAL AND LABORATORY STANDARDS INSTITUTE®

14th Edition

CLSI M02™

Performance Standards for Antimicrobial Disk Susceptibility Tests

CLSI M02 covers the current recommended methods for disk susceptibility testing and criteria for quality control testing.

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

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Performance Standards for Antimicrobial Disk Susceptibility Tests

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Abstract

Clinical and Laboratory Standards Institute M02—*Performance Standards for Antimicrobial Disk Susceptibility Tests* includes a series of procedures to standardize the way disk diffusion tests are performed. The performance, applications, and limitations of the current CLSI-recommended methods are also described.

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. In many medical microbiology laboratories, a disk diffusion method is used routinely for testing common, rapidly growing, and certain fastidious bacterial pathogens.

The supplemental information (CLSI M100[1] tables) used with this standard represents the most current information for drug selection, interpretation, and QC using the procedures standardized in CLSI M02.

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Foreword

The most current edition of CLSI M100,[1] 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,[2] and M11.[3]

Many other editorial and procedural changes in this edition of CLSI M02 resulted from Subcommittee on Antimicrobial Susceptibility Testing meetings held since 2018. Specific changes to the tables are summarized at the beginning of CLSI M100.[1] The most important changes in CLSI M02 are summarized below.

Overview of Changes

This standard replaces CLSI M02-Ed13, published in 2018. Several changes were made to 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"
- **Background:**
  - Added information on US Food and Drug Administration disk clearance
- **Subchapter 1.4.1, Definitions:**
  - Added definitions for antimicrobial susceptibility testing, disk diffusion, EDTA-modified carbapenem inactivation method, heteroresistance, and reference method
  - Revised definitions for breakpoint, D-zone test, supplemental (not routine) test, and susceptible-dose dependent
- **Subchapter 1.4.2, Abbreviations and Acronyms:**
  - Added abbreviations for Mueller-Hinton fastidious and staphylococci other than *S. aureus*
  - Deleted abbreviation for coagulase-negative staphylococci
- **Subchapter 2.1, Selecting Antimicrobial Agents for Routine Testing and Reporting:**
  - Moved former Subchapter 2.1 Selection Guidelines, to new Subchapter 2.1, Selecting Antimicrobial Agents for Routine Testing and Reporting, with clarified and updated information on selecting 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[1] 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[1] Tables 1
  - Added new Table 3, Antimicrobial Agent Test and Report Designations and Additional Considerations for Agents Not Listed in CLSI M100[1] Tables 1
  - Deleted Subchapter 2.3.1, β-Lactams
  - Deleted Subchapter 2.3.2, Non-β-Lactams

- **Subchapter 2.4, Antimicrobial Classes:**
  - Added new Table 4, Antimicrobial Agent Class Characteristics
- **Subchapter 3.1, Disk Diffusion Test Reagents:**
  - Added information on cefiderocol disk diffusion testing
- **Subchapter 3.4.2, Colony Suspension Method for Inoculum Preparation:**
  - Revised colony suspension method for inoculum preparation
- **Subchapter 3.4.4, Performing Colony Counts:**
  - Added new subchapter with instructions for performing colony counts in step-action table
- **Subchapter 3.7, Reading Plates and Interpreting Results:**
  - Revised process for testing *Staphylococcus* spp. with oxacillin disk
- **Subchapter 3.8, Special Considerations for Fastidious Organisms:**
  - Added information on Mueller-Hinton fastidious agar for *Haemophilus influenzae* and *Streptococcus pneumoniae* disk diffusion testing
- **Subchapter 3.9.1, Special Considerations for Detecting Resistance in Staphylococci:**
  - Added information on *mecC* isolates
  - 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.
  - Revised information on vancomycin-resistant *S. aureus,* vancomycin-intermediate *S. aureus,* and the vancomycin agar screen for *S. aureus*
  - Deleted former Subchapter 3.9.1.5, Linezolid Resistance, and instructions for reading linezolid disk diffusion test with transmitted light
- **Subchapter 3.9.2, Special Considerations for Detecting Resistance in Enterococci:**
  - Added information on detecting enterococci resistance with vancomycin agar screen test
- **Subchapter 3.9.4, Special Considerations for Detecting Resistance in Gram-Negative Bacilli:**
  - Added additional examples of β-lactamases in gram-negative bacteria to Table 8, Enzyme Classifications for β-Lactamases
  - Added additional examples of β-lactamases with carbapenemase activity to Table 9, β-Lactamases With Carbapenemase Activity
  - Revised information on AmpC enzymes and carbapenemases
- **Subchapter 3.11.3, Development of Resistance and Testing Repeat Isolates:**
  - Revised information on development of resistance
- **Subchapter 4.3, Selecting Strains for Quality Control:**
  - Revised information on selecting QC strains

- **Appendix A, Preparation of Media and Reagents:**
  - Added instructions for checking pH of GC agar to step-action table in section A1.3, GC Agar + 1% Defined Growth Supplement
  - Added instructions for checking pH of *Haemophilus* test medium agar to step-action table in section A1.4, *Haemophilus* Test Medium Agar
  - Added instructions for preparing Mueller-Hinton fastidious agar for testing *H. influenzae* or *S. pneumoniae* to new section A1.5, Mueller-Hinton Fastidious Agar
- **Appendix B, Conditions for Disk Diffusion Antimicrobial Susceptibility Tests:**
  - Added Mueller-Hinton fastidious agar for testing *H. influenzae* and *S. pneumoniae* to Table B2, Conditions for Disk Diffusion Antimicrobial Susceptibility Tests for Fastidious Organisms
- **Appendix E, Test for Performing Disk Diffusion Directly From Positive Blood Culture Broth:**
  - Added a procedure for performing disk diffusion directly from positive blood culture broth

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.[4]

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

antibiotic

antimicrobial agents

disk diffusion

susceptibility testing

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Chapter 1

Introduction

Performance Standards for Antimicrobial Disk Susceptibility Tests

1 Introduction

1.1 Scope

This standard describes the reference disk diffusion method used to determine the *in vitro* antimicrobial susceptibility of bacteria that grow aerobically and includes:

- Agar plate preparation
- Testing conditions, including inoculum preparation and standardization, incubation time, and incubation temperature
- Results interpretation
- QC procedures
- Disk diffusion 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 dilution methods are found in CLSI M07.[2] Standards for testing the *in vitro* antimicrobial susceptibility of bacteria that grow anaerobically are found in CLSI M11.[3] Guidelines for standardized antimicrobial susceptibility testing (AST) of infrequently isolated or fastidious bacteria that are not included in CLSI M02, M07,[2] or M11[3] are available in CLSI M45.[5] 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

Various laboratory methods can be used to measure the *in vitro* susceptibility of bacteria to antimicrobial agents. In many medical microbiology laboratories, a disk diffusion method is used routinely for testing common, rapidly growing, and certain fastidious bacterial pathogens. This standard describes the performance, applications, and limitations of the standardized disk diffusion test method. Recommendations[6] and governmental regulations[7,8] proposed by the US Food and Drug Administration (FDA) have been reviewed, and appropriate information is incorporated into this standard. Other AST methods, particularly disk diffusion reading devices, exist that provide results essentially equivalent to the CLSI methods described herein. The FDA is responsible for the clearance of antimicrobial agent disks and for the approval of commercial devices used in the United States, including specific devices for disk testing such as zone readers. CLSI does not approve or endorse commercial products or devices.