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13th Edition

M02

Performance Standards for Antimicrobial Disk Susceptibility Tests

SAMPLE

This standard 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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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. In many medical microbiology laboratories, an agar disk diffusion method is used routinely for testing common, rapidly growing, and certain fastidious bacterial pathogens. Clinical and Laboratory Standards Institute standard 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.

The supplemental information (M100¹ tables) used with this standard represents the most current information for drug selection, interpretation, and quality control using the procedures standardized in M02.

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Contents

| | |
|--|-----------|
| Abstract | i |
| Committee Membership | iii |
| Foreword | xi |
| Summary of CLSI Processes for Establishing Breakpoints and Quality Control Ranges | xiii |
| CLSI Reference Methods vs Commercial Methods and CLSI vs US Food and Drug Administration Breakpoints | xiv |
| Subcommittee on Antimicrobial Susceptibility Testing Mission Statement | xv |
| Chapter 1: Introduction | 1 |
| 1.1 Scope | 1 |
| 1.2 Background | 1 |
| 1.3 Standard Precautions | 2 |
| 1.4 Terminology | 2 |
| Chapter 2: Indications for Performing Antimicrobial Susceptibility Tests | 7 |
| 2.1 Selecting Antimicrobial Agents for Routine Testing and Reporting | 7 |
| 2.2 Routine Reports | 8 |
| 2.3 Antimicrobial Agent Classes | 8 |
| 2.4 Selection Guidelines | 12 |
| 2.5 Suggested Guidelines for Routine and Selective Testing and Reporting | 12 |
| Chapter 3: Disk Diffusion Antimicrobial Susceptibility Testing Process | 15 |
| 3.1 Disk Diffusion Test Reagents | 17 |
| 3.2 Testing Strains That Fail to Grow Satisfactorily | 18 |
| 3.3 Antimicrobial Disks | 18 |
| 3.4 Preparing Inoculum for Disk Diffusion Tests | 19 |
| 3.5 Inoculating the Test Plates | 21 |
| 3.6 Applying Disks to Inoculated Agar Plates | 22 |
| 3.7 Reading Plates and Interpreting Results | 22 |
| 3.8 Special Considerations for Fastidious Organisms | 24 |
| 3.9 Special Considerations for Detecting Resistance | 28 |
| 3.10 Supplemental (Not Routine) Tests | 37 |
| 3.11 Disk Diffusion Method Limitations | 39 |
| Chapter 4: Quality Control and Quality Assurance | 41 |
| 4.1 Quality Control Purpose | 41 |
| 4.2 Quality Control Responsibilities | 42 |
| 4.3 Selecting Strains for Quality Control | 42 |
| 4.4 Maintaining and Testing Quality Control Strains | 43 |
| 4.5 Batch or Lot Quality Control | 43 |
| 4.6 Zone Diameter Quality Control Ranges | 44 |
| 4.7 Quality Control Testing Frequency | 44 |
| 4.8 Out-of-Range Results With Quality Control Strains and Corrective Action | 46 |
| 4.9 Reporting Patient Results When Out-of-Range Quality Control Results Are Observed | 49 |
| 4.10 Confirming Results When Testing Patient Isolates | 49 |
| 4.11 End-Point Interpretation Control | 50 |

Contents (Continued)

| | |
|--|----|
| Chapter 5: Conclusion..... | 52 |
| Chapter 6: Supplemental Information..... | 52 |
| References..... | 53 |
| Appendix A. Preparation of Media and Reagents..... | 56 |
| Appendix B. Conditions for Disk Diffusion Antimicrobial Susceptibility Tests | 60 |
| Appendix C. Quality Control Strain Maintenance..... | 64 |
| Appendix D. Quality Control Protocol Flow Charts..... | 66 |
| The Quality Management System Approach..... | 70 |
| Related CLSI Reference Materials | 71 |

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Foreword

The most current edition of CLSI document 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 M02 and CLSI document M07.³

Many other editorial and procedural changes in this edition of M02 resulted from Subcommittee on Antimicrobial Susceptibility Testing meetings held since 2015. Specific changes to the tables are summarized at the beginning of M100.¹ The most important changes in M02 are summarized below.

Overview of Changes

This standard replaces the previous edition of the approved standard, M02-A12, published in 2015. Several changes were made in this edition, including:

- **General:**
 - Harmonized language and information on drug selection and QC with CLSI document M07³
 - 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 2.3.2.2, Folate Pathway Antagonists:**
 - Revised nomenclature from “folate pathway inhibitor” to “folate pathway antagonist”
- **Subchapter 2.3, Antimicrobial Agent Classes:**
 - Clarified and updated the antimicrobial agent classes
- **Subchapter 3.1.2, Handling and Storing Mueller-Hinton Agar Plates:**
 - Added information on proper storage of Mueller-Hinton agar plates
- **Subchapter 3.7, Reading Plates and Interpreting Results:**
 - Added reference to the *M02 Disk Diffusion Reading Guide*²
 - Added instruction to read vancomycin results for *Enterococcus* with transmitted light
- **Subchapter 3.8, Table 1. Testing Considerations for Fastidious Organisms:**
 - Clarified source plate incubation times and inoculum broth for some fastidious organisms
- **Subchapter 3.9, Special Considerations for Detecting Resistance:**
 - Reorganized and streamlined

Performance Standards for Antimicrobial Disk Susceptibility Tests

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 the reference agar 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, 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 dilution methods are found in CLSI document M07.³ 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, M07,³ or M11⁶ 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

Various laboratory methods can be used to measure the *in vitro* susceptibility of bacteria to antimicrobial agents. In many medical microbiology laboratories, an agar 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. Recommendations⁸ and governmental regulations^{9,10} proposed by the US Food and Drug Administration

(FDA) have been reviewed, and appropriate information was incorporated into this standard. Other AST methods 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.

Disk diffusion tests based solely on the presence or absence of a zone of inhibition without regard to the zone's size are not acceptable for determining antimicrobial susceptibility. Reliable results can only be obtained with disk diffusion tests that use 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 original and most thoroughly described disk diffusion method.¹¹ This method is the most thoroughly described disk diffusion method for which breakpoints and interpretive categories have been developed and supported by laboratory and clinical data.

This standard, along with M100,¹ describes methods, QC, breakpoints, and interpretive categories currently recommended for disk diffusion susceptibility tests. For most antimicrobial agents, these criteria are developed by first comparing zone diameters to MICs for a large number of isolates, including those with known resistance mechanisms relevant to the particular drug class. Second, the MICs and correlated zone sizes are analyzed in relation to the pharmacokinetics of the drug from normal dosage regimens. Finally, when feasible, *in vitro* breakpoints are analyzed in relation to studies of clinical efficacy and microbiological eradication efficacy in the treatment of specific pathogens, as outlined in CLSI document M23.⁵

When new problems are recognized or improvements in these criteria are developed, changes will be incorporated into future editions of this standard and M100.¹

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.¹² 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 M29.¹³

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**.

The Quality Management System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management system (QMS) approach in the development of standards and guidelines that facilitates project management, defines a document structure using a template, and provides a process to identify needed documents. The QMS approach applies a core set of “quality system essentials” (QSEs), basic to any organization, to all operations in any health care service’s path of workflow (ie, operational aspects that define how a particular product or service is provided). The QSEs provide the framework for delivery of any type of product or service, serving as a manager’s guide. The QSEs are:

| | | | |
|-----------------------|--------------------------|------------------------|--------------------------------|
| Organization | Personnel | Process Management | Nonconforming Event Management |
| Customer Focus | Purchasing and Inventory | Documents and Records | Assessments |
| Facilities and Safety | Equipment | Information Management | Continual Improvement |

M02 covers the QSE indicated by an “X.” For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section.

| Organization | Customer Focus | Facilities and Safety | Personnel | Purchasing and Inventory | Equipment | Process Management | Documents and Records | Information Management | Nonconforming Event Management | Assessments | Continual Improvement |
|--------------|----------------|-----------------------|-----------|--------------------------|-----------|---|-----------------------|------------------------|--------------------------------|-------------|-----------------------|
| | | M29 | | | | X EP23 M07 M11 M23 M45 M100 | | | | | |

Path of Workflow

A path of workflow is the description of the necessary processes to deliver the particular product or service that the organization or entity provides. A laboratory path of workflow consists of the sequential processes: preexamination, examination, and postexamination and their respective sequential subprocesses. All laboratories follow these processes to deliver their services, namely quality laboratory information.

M02 covers the medical laboratory path of workflow processes indicated by an “X.” For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section.

| Preexamination | | | Examination | | | Postexamination | | |
|----------------------|-------------------|------------------|-------------------------------|-------------------------|--|--|---------------------------------|-------------------|
| Examination ordering | Sample collection | Sample transport | Sample receipt and processing | Examination | Results review and follow-up | Interpretation | Results reporting and archiving | Sample management |
| | | | | X EP23 M07 M11 | X EP23 M07 M11 M45 M100 | X EP23 M07 M11 M45 M100 | X M07 M11 M45 M100 | |

Related CLSI Reference Materials*

- EP23™** **Laboratory Quality Control Based on Risk Management. 1st ed., 2011.** This document provides guidance based on risk management for laboratories to develop quality control plans tailored to the particular combination of measuring system, laboratory setting, and clinical application of the test.
- M02QG** **Disk Diffusion Reading Guide. 1st ed., 2018.** The Disk Diffusion Reading Guide provides photographic examples of the proper method for reading disk diffusion susceptibility testing results.
- M07** **Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. 11th ed., 2018.** This standard covers reference methods for determining minimal inhibitory concentrations of aerobic bacteria by broth macrodilution, broth microdilution, and agar dilution.
- M11** **Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria. 8th ed., 2012.** This standard provides reference methods for the determination of minimal inhibitory concentrations of anaerobic bacteria by agar dilution and broth microdilution.
- M23** **Development of *In Vitro* Susceptibility Testing Criteria and Quality Control Parameters. 5th ed., 2018.** This guideline discusses the necessary and recommended data for selecting appropriate breakpoints and quality control ranges for antimicrobial agents.
- M29** **Protection of Laboratory Workers From Occupationally Acquired Infections. 4th ed., 2014.** Based on US regulations, this document provides guidance on the risk of transmission of infectious agents by aerosols, droplets, blood, and body substances in a laboratory setting; specific precautions for preventing the laboratory transmission of microbial infection from laboratory instruments and materials; and recommendations for the management of exposure to infectious agents.
- M45** **Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria. 3rd ed., 2016.** This guideline informs clinical, public health, and research laboratories on susceptibility testing of infrequently isolated or fastidious bacteria that are not included in CLSI documents M02, M07, or M100. Antimicrobial agent selection, test interpretation, and quality control are addressed.
- M100** **Performance Standards for Antimicrobial Susceptibility Testing. 28th ed., 2018.** This document includes updated tables for the Clinical and Laboratory Standards Institute antimicrobial susceptibility testing standards M02, M07, and M11.

* CLSI documents are continually reviewed and revised through the CLSI consensus process; therefore, readers should refer to the most current editions.

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