CLINICAL AND LABORATORY STANDARDS INSTITUTE®

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M02-A12

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

This standard 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.

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

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Abstract

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.

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. Clinical and Laboratory Standards Institute document M02-A12—*Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Twelfth Edition* 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 (M100[1] tables) presented with this standard represents the most current information for drug selection, interpretation, and QC using the procedures standardized in M02. These tables, as in previous years, have been updated and should replace tables published in earlier years. Changes in the tables since the previous edition (M100-S24) appear in boldface type and are also summarized in the front of the document.

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Foreword

In this revision of M02, several sections were added or revised as outlined below in the Summary of Changes. One of the main updates is the reformatting of the document to follow a laboratory's path of workflow—defined as the sequential processes of preexamination, examination, and postexamination. An overview of the disk diffusion susceptibility testing process is provided in the beginning of the document in the new Figure 1 (see Chapter 3) with various testing methods shown in easy-to-follow step-action tables throughout the document.

The most current edition of CLSI document M100,[1] published as an annual volume of tables, 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 presented in the annual tables.

Many other editorial and procedural changes in this edition of M02 resulted from meetings of the Subcommittee on Antimicrobial Susceptibility Testing since 2012. Specific changes to the M100[1] tables are summarized at the beginning of CLSI document M100.[1] The most important changes in M02 are summarized below.

Summary of Changes

Formatting Changes Throughout the Document:

- Main sections are now referred to as "Chapters." Sections within the chapters are referred to as "Subchapters."

- Easy-to-follow step-action tables are introduced, consistent with CLSI's goal to make standards and guidelines more user friendly. Most of these tables strictly reflect reformatting of text that previously appeared in M02. Any changes to the testing recommendations are highlighted here in the Summary of Changes. The new step-action tables within the document include:
  - Subchapter 3.3.2, Storage of Antimicrobial Disks
  - Subchapter 3.4.2, Direct Colony Suspension Method for Inoculum Preparation
  - Subchapter 3.4.3, Growth Method for Inoculum Preparation
  - Subchapter 3.5, Inoculation of Test Plates
  - Subchapter 3.6, Application of Disks to Inoculated Agar Plates
  - Subchapter 3.9.1.7.2, Vancomycin Agar Screen (*Staphylococcus aureus*)
  - Subchapter 3.9.2.3, Vancomycin Agar Screen (*Enterococcus* spp.)

Subchapter 1.4.1, Definitions
Added definitions for susceptible-dose dependent, test method, and test system.

Expanded the definition of quality control.

Subchapter 2.3, Suggested Guidelines for Routine and Selective Testing and Reporting
Provided additional information on the location of Test and Report Group designations in M100.[1]

Noted cefazolin is a surrogate agent in Test and Report Group U and is not reported exclusively on urine isolates.

Chapter 3, Susceptibility Testing Process
Added a flow chart that provides an overview of the disk diffusion susceptibility testing process.

Subchapter 3.6, Application of Disks to Inoculated Agar Plates
Modified recommendation from "5" to "6 or fewer" as the number of disks that can be placed on a 100-mm plate.

Subchapter 3.7, Special Considerations for Fastidious Organisms
Added table that summarizes special testing requirements (eg, media, incubation time, and temperature) for fastidious organisms.

Subchapter 3.8, Reading Plates and Interpreting Results
Clarified time of incubation for testing of cefoxitin against *Staphylococcus* spp.: 24 hours for coagulase-negative *Staphylococcus* spp.; 16 to 18 hours for *S. aureus.*

Noted that the penicillin zone edge test can be useful for determining β-lactamase production in *S. aureus* strains with penicillin zones ≥ 29 mm.

Added susceptible-dose dependent to the list of disk diffusion and minimal inhibitory concentration (MIC) interpretive categories.

Subchapter 3.9.1.2, Methicillin/Oxacillin Resistance
Expanded explanation of mechanisms and generic determinants of oxacillin resistance in staphylococci, which includes *mecC* in *S. aureus.*

Subchapter 3.9.1.4, Methods for Detection of Oxacillin Resistance
Expanded the discussion of oxacillin resistance and added a table that summarizes the tests available to detect oxacillin resistance in staphylococci.

Subchapter 3.9.1.6, Reporting
Clarified several reporting recommendations to include: application of oxacillin results to other penicillinase-stable penicillins and reporting results for *mecA*- and/or penicillin-binding protein 2a–negative *S. aureus* with oxacillin MICs ≥ 4 µg/mL.

Subchapter 3.9.1.7.4, Reporting
Further emphasized the need to confirm and communicate results to appropriate authorities when *S. aureus* and coagulase-negative staphylococci with vancomycin MICs of ≥ 8 µg/mL and ≥ 32 µg/mL, respectively, are encountered.

Subchapter 3.9.1.10, Mupirocin Resistance
Noted that use of mupirocin is known to increase rates of high-level mupirocin resistance in *S. aureus.*

Subchapter 3.9.2.4, High-Level Aminoglycoside Resistance
Noted that high-level resistance to both gentamicin and streptomycin implies resistance to all aminoglycosides.

Subchapter 3.9.3.1, Extended-Spectrum β-Lactamases
Updated discussion of extended-spectrum β-lactamases.

Subchapter 3.9.3.3, Carbapenemases (Carbapenem-Resistant Gram-Negative Bacilli)
Added reference to the Carba NP colorimetric microtube assay to detect carbapenemase activity.

Subchapter 3.10.1, Inducible Clindamycin Resistance
Noted that infections due to streptococci with inducible clindamycin resistance may fail to respond to clindamycin therapy.

Subchapter 4.3, Selection of Strains for Quality Control
Expanded description of routine and supplemental QC strains.

Subchapter 4.4, Maintenance and Testing of Quality Control Strains
Introduced terms "F1," "F2," and "F3" to relate to "frozen" or "freeze-dried" subcultures of QC strains and provided enhanced recommendations for handling QC strains.

Subchapter 4.7.2, Performance Criteria for Reducing Quality Control Frequency to Weekly
Introduced for the first time in M02 the 15-replicate (3 × 5 day) QC plan as an alternative to the 20- or 30-day QC plan.

Appendix A, Quality Control Protocol Flow Charts
Revised and expanded flow charts to better convey the QC testing process and added flow charts that depict the new 15-replicate (3 × 5 day) QC option to convert from daily to weekly QC testing.

Appendix E, Quality Control Strain Maintenance
Revised schematic that depicts stages of subculture and testing of QC strains that originate from "frozen" or "freeze-dried" stock cultures.

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

Chapter 1: Introduction

This chapter includes:

- Document scope and applicable exclusions
- Background information pertinent to the document content
- Standard precautions information
- Terms and definitions used in the document
- Abbreviations and acronyms used in the document

1.1 Scope

This document describes the standard agar disk diffusion techniques used to determine the *in vitro* susceptibility of bacteria that grow aerobically. It addresses preparation of agar plates, testing conditions (including inoculum preparation and standardization, incubation time, and incubation temperature), interpretation of results, 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 using dilution methods are found in CLSI document M07[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, M07,[3] or M11[4] are available in CLSI document M45.[5]

The susceptibility testing 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

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[6] and regulations[7,8] proposed by the US Food and Drug Administration (FDA) have been reviewed, and appropriate sections were 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 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 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.[9] This method is the most thoroughly described disk diffusion method for which interpretive standards have been developed and supported by laboratory and clinical data.

This document, along with M100,[1] describes methods, QC, and interpretive criteria currently recommended for disk diffusion susceptibility tests. For most agents, these criteria are developed by first comparing zone diameters to MICs of a large number of isolates, including those with known mechanisms of resistance relevant to the particular class of drug. Second, the MICs and correlated zone sizes are analyzed in relation to the pharmacokinetics of the drug from normal dosing regimens. Finally, when feasible, *in vitro* interpretive criteria 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.[2]

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[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. The Centers for Disease Control and Prevention (CDC) address this topic in published guidelines that address the daily operations of diagnostic medicine in human and animal medicine while encouraging a culture of safety in the laboratory.[10] 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.[11]

1.4 Terminology

1.4.1 Definitions

antimicrobial susceptibility test interpretive category – a classification based on an *in vitro* response of an organism to an antimicrobial agent at levels corresponding to blood or tissue levels attainable with usually prescribed doses of that agent.

1) **susceptible (S)** – a category that implies that isolates are inhibited by the usually achievable concentrations of antimicrobial agent when the dosage recommended to treat the site of infection is used.

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, which facilitates project management; defines a document structure via 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 as follows:

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-A12 addresses 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 on the following page.

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 M06 M07 M11 M23 M27 M27-S4 M45	M07				

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 the laboratory's services, namely quality laboratory information.

M02-A12 addresses the clinical laboratory path of workflow steps indicated by an "X." For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

Preexamination				**Examination**			**Postexamination**	
Examination ordering	Sample collection	Sample transport	Sample receipt/processing	Examination	Results review and follow-up	Interpretation	Results reporting and archiving	Sample management
				X EP23 M07 M11 M27 M27-S4	X EP23 M07 M11 M27 M27-S4 M100	X EP23 M07 M11 M27 M27-S4 M100	X M07 M11 M27 M27-S4 M100	 M27 M27-S4

Related CLSI Reference Materials*

EP23-A™ **Laboratory Quality Control Based on Risk Management; Approved Guideline (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.

M06-A2 **Protocols for Evaluating Dehydrated Mueller-Hinton Agar; Approved Standard—Second Edition (2006).** This document provides procedures for evaluating production lots of dehydrated Mueller-Hinton agar, and for developing and applying reference media.

M07-A10 **Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Tenth Edition (2015).** This standard addresses reference methods for the determination of minimal inhibitory concentrations of aerobic bacteria by broth macrodilution, broth microdilution, and agar dilution.

M11-A8 **Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard—Eighth Edition (2012).** This standard provides reference methods for the determination of minimal inhibitory concentrations of anaerobic bacteria by agar dilution and broth microdilution.

M23-A3 **Development of *In Vitro* Susceptibility Testing Criteria and Quality Control Parameters; Approved Guideline—Third Edition (2008).** This document addresses the required and recommended data needed for the selection of appropriate interpretive criteria and quality control ranges for antimicrobial agents.

M27-A3 **Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard—Third Edition (2008).** This document addresses the selection and preparation of antifungal agents; implementation and interpretation of test procedures; and quality control requirements for susceptibility testing of yeasts that cause invasive fungal infections.

M27-S4 **Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Fourth Informational Supplement (2012).** This document provides updated tables for the CLSI antimicrobial susceptibility testing standard M27-A3.

M29-A4 **Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Fourth Edition (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-A2 **Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Approved Guideline—Second Edition (2010).** This document provides guidance to clinical microbiology laboratories for standardized susceptibility testing of infrequently isolated or fastidious bacteria that are not presently included in CLSI documents M02 or M07. The tabular information in this document presents the most current information for drug selection, interpretation, and quality control for the infrequently isolated or fastidious bacterial pathogens included in this guideline.

M100-S25 **Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement (2015).** This document provides updated tables for the Clinical and Laboratory Standards Institute antimicrobial susceptibility testing standards M02-A12, M07-A10, and M11-A8.

* 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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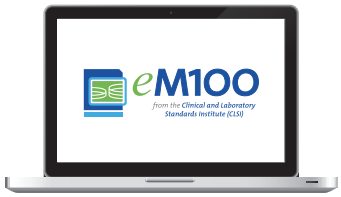

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