M07-A10
Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Tenth Edition

This standard addresses reference methods for the determination of 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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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. This document describes standard broth dilution (macrodilution and microdilution [the microdilution method described in 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 (M100 tables) presented with this standard represents the most current information for drug selection, interpretation, and QC using the procedures standardized in M07. 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 M07, 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 minimal inhibitory concentration (MIC) 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, 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 M07 resulted from meetings of the Subcommittee on Antimicrobial Susceptibility Testing since 2012. Specific changes to the M100 tables are summarized at the beginning of CLSI document M100. The most important changes in M07 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 M07. 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, Direct Colony Suspension Method for Inoculum Preparation
  - Subchapter 3.3.3, Growth Method for Inoculum Preparation
  - Subchapter 3.5, Preparing Agar Dilution Plates
  - Subchapter 3.5.7, Inoculating Agar Dilution Plates
  - Subchapter 3.5.8, Incubating Agar Dilution Plates
  - Subchapter 3.5.9, Determining Agar Dilution End Points
  - Subchapter 3.7, Macrodilution (Tube) Broth Method
  - Subchapter 3.8, Broth Microdilution Method
  - Subchapter 3.9, Incubation
  - Subchapter 3.11, Determining Broth Macro- or Microdilution End Points
  - Subchapter 3.13.1.7.2, Vancomycin Agar Screen (S. aureus)
  - Subchapter 3.13.2.3, 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.

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 MIC susceptibility testing process.

Subchapter 3.6.1, Mueller-Hinton Broth
Added information for preparation of cation-adjusted Mueller-Hinton broth or Mueller-Hinton agar when testing tigecycline and omadacycline.

Subchapter 3.12, Special Considerations for Fastidious Organisms
Added table that summarizes special testing requirements (eg, media, incubation time, and temperature) for fastidious organisms.

Subchapter 3.13.1.2, Methicillin/Oxacillin Resistance
Expanded explanation of mechanisms and genetic determinants of oxacillin resistance in staphylococci, which includes mecC in Staphylococcus aureus.

Subchapter 3.13.1.4, Methods for Detection of Oxacillin Resistance
Expanded discussion of oxacillin resistance and added a table that summarizes the tests available to detect oxacillin resistance in staphylococci.

Subchapter 3.13.1.6, 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 3.13.1.7.4, 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 3.13.1.9, Mupirocin Resistance
Noted that use of mupirocin is known to increase rates of high-level mupirocin resistance in S. aureus.

Subchapter 3.13.2.4, High-Level Aminoglycoside Resistance
Noted that high-level resistance to both gentamicin and streptomycin implies resistance to all aminoglycosides.

Subchapter 3.13.3.1, Extended-Spectrum β-Lactamases
Updated discussion of extended-spectrum β-lactamases.

Subchapter 3.13.3.3, Carbapenemases (Carbapenem-Resistant Gram-Negative Bacilli)
Added reference to the Carba NP colorimetric microtube assay to detect carbapenemase activity.

Subchapter 3.14.1, 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.
Subchapter 4.7.2, Performance Criteria for Reducing Quality Control Frequency to Weekly
Introduced for the first time in M07 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.
Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Tenth 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 broth (macrodilution and microdilution) and agar dilution methods used to determine the \textit{in vitro} susceptibility of bacteria that grow aerobically. It addresses preparation of broth and agar dilution tests, testing conditions (including inoculum preparation and standardization, incubation time, and incubation temperature), reporting of minimal inhibitory concentration (MIC) results, QC procedures, and limitations of the dilution test methods. To assist the clinical laboratory, suggestions are provided on the selection of antimicrobial agents for routine testing and reporting.

Standards for testing the \textit{in vitro} susceptibility of bacteria that grow aerobically using the antimicrobial disk susceptibility testing method are found in CLSI document M02; standards for testing the \textit{in vitro} susceptibility of bacteria that grow anaerobically are found in CLSI document M11. Guidelines for standardized susceptibility testing of infrequently isolated or fastidious bacteria that are not included in CLSI documents M02, M07, or M11 are available in CLSI document M45.

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

Either broth or agar dilution methods may be used to measure quantitatively the \textit{in vitro} activity of an antimicrobial agent against a given bacterial isolate. To perform the tests, a series of tubes or plates is prepared with a broth or agar medium to which various concentrations of the antimicrobial agents are added. The tubes or plates are then inoculated with a standardized suspension of the test organism. After incubation at 35°C ± 2°C, the tests are examined and the MIC is determined. The final result is significantly influenced by methodology, which must be carefully controlled if reproducible results (intralaboratory and interlaboratory) are to be achieved.

This document describes standard reference broth dilution (macrodilution and microdilution) and agar dilution methods. The basics of these methods are derived, in large part, from information generated by the
International Collaborative Study. Although these methods are standard reference methods, some are sufficiently practical for routine use in both clinical laboratories and research 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 here. The US Food and Drug Administration (FDA) is responsible for the approval of commercial devices used in the United States. CLSI does not approve or endorse commercial products or devices.

The methods described in this document 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 3.12 and M100\(^2\) Tables 2E through 2I. Methods for testing anaerobic bacteria are outlined in CLSI document M11\(^5\) and in M100\(^2\) Table 2J. Methods for testing infrequently isolated or fastidious bacteria not included in CLSI documents M02\(^4\) and M07 are found in CLSI document M45.\(^6\)

This document, along with M100,\(^2\) describes methods, QC, and interpretive criteria 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 also distributed in annual informational supplements (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. 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. 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.\(^9\)

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.

2) susceptible-dose dependent (SDD) – a category that implies that susceptibility of an isolate is dependent on the dosing regimen that is used in the patient. In order to achieve levels that are likely to be clinically effective against isolates for which the susceptibility testing results (either minimal inhibitory concentrations [MICs] or disk diffusion) are in the SDD category, it is necessary to use a dosing regimen (ie, higher doses, more frequent doses, or both) that results in higher drug exposure than the dose that was used to establish the susceptible breakpoint. Consideration should be given to

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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 (i.e., 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
- Customer Focus
- Personnel
- Purchasing and Inventory
- Process Management
- Nonconforming Event Management
- Facilities and Safety
- Equipment
- Documents and Records
- Information Management
- Assessments
- Continual Improvement

M07-A10 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.

### 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.

M07-A10 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.
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.

**M02-A12** Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Twelfth Edition (2015). 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.


**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.


**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.

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