CLINICAL AND LABORATORY STANDARDS INSTITUTE®

11th Edition

M07

Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically

This standard 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.

Clinical and Laboratory Standards Institute

Setting the standard for quality in medical laboratory testing around the world.

The Clinical and Laboratory Standards Institute (CLSI) is a not-for-profit membership organization that brings together the varied perspectives and expertise of the worldwide laboratory community for the advancement of a common cause: to foster excellence in laboratory medicine by developing and implementing medical laboratory standards and guidelines that help laboratories fulfill their responsibilities with efficiency, effectiveness, and global applicability.

Consensus Process

Consensus—the substantial agreement by materially affected, competent, and interested parties—is core to the development of all CLSI documents. It does not always connote unanimous agreement but does mean that the participants in the development of a consensus document have considered and resolved all relevant objections and accept the resulting agreement.

Commenting on Documents

CLSI documents undergo periodic evaluation and modification to keep pace with advances in technologies, procedures, methods, and protocols affecting the laboratory or health care.

CLSI's consensus process depends on experts who volunteer to serve as contributing authors and/or as participants in the reviewing and commenting process. At the end of each comment period, the committee that developed the document is obligated to review all comments, respond in writing to all substantive comments, and revise the draft document as appropriate.

Comments on published CLSI documents are equally essential and may be submitted by anyone, at any time, on any document. All comments are managed according to the consensus process by a committee of experts.

Appeal Process

When it is believed that an objection has not been adequately considered and responded to, the process for appeal, documented in the CLSI *Standards Development Policies and Processes,* is followed.

All comments and responses submitted on draft and published documents are retained on file at CLSI and are available upon request.

Get Involved—Volunteer!

Do you use CLSI documents in your workplace? Do you see room for improvement? Would you like to get involved in the revision process? Or maybe you see a need to develop a new document for an emerging technology? CLSI wants to hear from you. We are always looking for volunteers. By donating your time and talents to improve the standards that affect your own work, you will play an active role in improving public health across the globe.

For additional information on committee participation or to submit comments, contact CLSI.

Clinical and Laboratory Standards Institute
950 West Valley Road, Suite 2500
Wayne, PA 19087 USA
P: +1.610.688.0100
F: +1.610.688.0700
www.clsi.org
standard@clsi.org

M07, 11th ed.
January 2018
Replaces M07-A10

Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically

Melvin P. Weinstein, MD
Jean B. Patel, PhD, D(ABMM)
Carey-Ann Burnham, PhD, D(ABMM)
Shelley Campeau, PhD, D(ABMM)
Patricia S. Conville, MS, MT(ASCP)
Christopher Doern, PhD, D(ABMM)
George M. Eliopoulos, MD
Marcelo F. Galas
Romney M. Humphries, PhD, D(ABMM)
Stephen G. Jenkins, PhD, D(ABMM), F(AAM)
Susan M. Kircher, MS, MT(ASCP)
James S. Lewis II, PharmD, FIDSA
Brandi Limbago, PhD
Amy J. Mathers, MD, D(ABMM)
Tony Mazzulli, MD, FACP, FRCP(C)
Susan D. Munro, CLS, MT(ASCP)
Margaret Ordoñez Smith de Danies, PhD
Robin Patel, MD
Sandra S. Richter, MD, D(ABMM), FCAP, FIDSA
Michael Satlin, MD, MS
Jana M. Swenson, MMSc
Alexandra Wong, BS, MT(ASCP), SM
Wayne F. Wang, MD, PhD
Barbara L. Zimmer, PhD

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. Clinical and Laboratory Standards Institute standard M07—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically* describes standard broth dilution (macrodilution and microdilution [the microdilution method described in M07 is the same methodology outlined in ISO 20776-1[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[2] tables) used with this standard represents the most current information for drug selection, interpretation, and quality control using the procedures standardized in M07.

Clinical and Laboratory Standards Institute (CLSI). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07 (ISBN 1-56238-836-3 [Print]; ISBN 1-56238-837-1 [Electronic]). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2018.

Suggested Citation

CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically.* 11th ed. CLSI standard M07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

Previous Editions:
June 1980, December 1982, June 1986, November 1988, April 1990, December 1993, January 1997, January 2000, January 2003, January 2006, January 2009, January 2012, January 2015

ISBN 1-56238-836-3 (Print)
ISBN 1-56238-837-1 (Electronic)
ISSN 1558-6502 (Print)
ISSN 2162-2914 (Electronic) Volume 38, Number 2

Contents

Contents (Continued)

Foreword

The most current edition of CLSI document M100,[2] 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 M07 and CLSI document M02.[3]

Many other editorial and procedural changes in this edition of M07 resulted from Subcommittee on Antimicrobial Susceptibility Testing meetings held since 2015. Specific changes to the tables are summarized at the beginning of M100.[2] The most important changes in M07 are summarized below.

Overview of Changes

This standard replaces the previous edition of the approved standard, M07-A10, published in 2015. Several changes were made in this edition, including:

- **General:**
  - Harmonized language and information on drug selection and QC with CLSI document M02[3]
  - 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, Antimicrobial Agent Classes:**
  - Clarified and updated antimicrobial agent classes
- **Subchapter 2.3.2.2, Folate Pathway Antagonists:**
  - Revised nomenclature from "folate pathway inhibitor" to "folate pathway antagonist"
- **Subchapter 3.9, Determining Broth Macro- or Microdilution End Points:**
  - Added photographs of growth control examples and for interpreting skipped wells
- **Subchapter 3.11, Table 1. Testing Considerations for Fastidious Organisms:**
  - Clarified source plate incubation times and inoculum broth for some fastidious organisms
- **Subchapter 3.12, Special Considerations for Detecting Resistance:**
  - Reorganized and streamlined
  - Moved Subchapters 3.12.4 (Inducible Clindamycin Resistance) and 3.12.6 (β-Lactamase Tests) to create a new subchapter, 3.13 (Supplemental [Not Routine] Tests)

Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically

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 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
- Reporting minimal inhibitory concentration (MIC) results
- QC procedures
- Dilution test 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 the antimicrobial disk testing method are found in CLSI document M02.[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,[3] M07, or M11[6] 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

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, plates or a series of tubes are prepared with an agar or broth medium to which various concentrations of the antimicrobial agents are added. The plates or tubes are then inoculated with a standardized suspension of the test organism. After incubating for the appropriate time interval, the tests are read, the 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.

This standard describes reference broth dilution (macrodilution and microdilution) and agar dilution methods. The basic components of these methods are largely derived from information contained in published recommendations.[8] Although these methods are standard reference methods, some are sufficiently practical for routine use in medical or public health 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. CLSI does not approve or endorse commercial products or devices.

The methods described in this standard 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 3.11 and M100[2] Tables 2E through 2I. Methods for testing anaerobic bacteria are provided in CLSI document M11[6] and in M100[2] Table 2J. Methods for testing infrequently isolated or fastidious bacteria not included in CLSI documents M02[3] and M07 are found in CLSI document M45.[7]

This standard, along with M100,[2] describes methods, QC, breakpoints, and interpretive categories 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 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. 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.[9] 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.[10]

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

M07 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 M02 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.

M07 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 M02 M11	X EP23 M02 M11 M45 M100	X EP23 M02 M11 M45 M100	X M02 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.

M02 **Performance Standards for Antimicrobial Disk Susceptibility Tests. 13th ed., 2018.** This standard covers the current recommended methods for disk susceptibility testing and criteria for quality control testing.

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.

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

Explore the Latest Offerings From CLSI!

As we continue to set the global standard for quality in laboratory testing, we are adding products and programs to bring even more value to our members and customers.

By becoming a CLSI member, your laboratory will join 1,600+ other influential organizations all working together to further CLSI's efforts to improve health care outcomes. You can play an active role in raising global laboratory testing standards—in your laboratory, and around the world.

Find out which membership option is best for you at **www.clsi.org/membership.**

Find what your laboratory needs to succeed! CLSI U provides convenient, cost-effective continuing education and training resources to help you advance your professional development. We have a variety of easy-to-use, online educational resources that make *e*Learning stress-free and convenient for you and your staff.

See our current educational offerings at **www.clsi.org/education.**

When laboratory testing quality is critical, standards are needed and there is no time to waste. eCLIPSE™ Ultimate Access, our cloud-based online portal of the complete library of CLSI standards, makes it easy to quickly find the CLSI resources you need.

Learn more and purchase eCLIPSE at **clsi.org/eCLIPSE.**

For more information, visit www.clsi.org today.

CLINICAL AND
LABORATORY
STANDARDS
INSTITUTE®

950 West Valley Road, Suite 2500, Wayne, PA 19087 USA

P: +1.610.688.0100 **Toll Free (US):** 877.447.1888 **F:** +1.610.688.0700

E: customerservice@clsi.org **www.clsi.org**

PRINT ISBN 1-56238-836-3

ELECTRONIC ISBN 1-56238-837-1