



CLINICAL AND  
LABORATORY  
STANDARDS  
INSTITUTE®

9th Edition

# M11

## Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria

This standard provides reference methods for determining minimal inhibitory concentrations of anaerobic bacteria by agar dilution and broth microdilution.

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

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# Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria

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## Abstract

Clinical and Laboratory Standards Institute standard M11—*Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria* describes the reference standard agar dilution method (Wadsworth) and the alternative broth microdilution method. Antimicrobial resistance patterns for many anaerobic bacteria have changed significantly over the past several years, resulting in a lack of predictability for many species. Susceptibility testing of anaerobes is recommended for surveillance purposes and for specific clinical situations. The agar dilution method is well suited for surveillance testing and research. It is also the standard with which other methods are compared. The alternative method, broth microdilution, is well suited for the medical laboratory but is currently limited to testing *Bacteroides* spp. and *Parabacteroides* spp. organisms and selected antimicrobial agents. QC criteria for each procedure are also described. This standardized procedure, when used in conjunction with the M100<sup>1</sup> tables, includes the most current information for drug selection, interpretation, QC, and antibiogram reports. When new problems are recognized or improvements in these criteria are made, changes will be incorporated into future editions of this standard and in M100.<sup>1</sup>

Clinical and Laboratory Standards Institute (CLSI). *Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria*. 9th ed. CLSI standard M11 (ISBN 978-1-68440-021-8 [Print]; ISBN 978-1-68440-022-5 [Electronic]). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2018.

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### **Suggested Citation**

CLSI. *Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria*. 9th ed. CLSI standard M11. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

### **Previous Editions:**

November 1979, January 1982, June 1984, April 1989, August 1990, December 1993, December 1997, March 2001, January 2004, January 2007, February 2012

ISBN 978-1-68440-021-8 (Print)  
ISBN 978-1-68440-022-5 (Electronic)  
ISSN 1558-6502 (Print)  
ISSN 2162-2914 (Electronic)

Volume 38, Number 19

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## Acknowledgment for the Expert Panel on Microbiology

CLSI, the Consensus Council, and the Subcommittee on Antimicrobial Susceptibility Testing gratefully acknowledge the Expert Panel on Microbiology for serving as technical advisors and subject matter experts during the development of this standard.

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## Acknowledgment

CLSI, the Consensus Council, and the Subcommittee on Antimicrobial Susceptibility Testing gratefully acknowledge the following volunteers for their important contributions to the development of this standard:

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## Foreword

High-level antimicrobial agent resistance among anaerobic organisms is continually reported.<sup>2-6</sup> Resistance rates vary among species and from institution to institution.<sup>5</sup> Even within the same species, minimal inhibitory concentrations (MICs) to particular agents may vary significantly.<sup>7</sup> Among *Bacteroides* spp., resistance to some commonly used antimicrobial agents (eg, clindamycin, moxifloxacin) can be high. Significant variability can occur among isolates from hospitalized patients in multiple institutions in the same geographic region. In addition, resistance has been reported among the most active drugs, such as imipenem, piperacillin-tazobactam, tigecycline, ampicillin-sulbactam, and metronidazole.<sup>6,8-11</sup>

Significant resistance rates are also identified in many non-*Bacteroides* anaerobe species, including *Prevotella* spp., *Peptostreptococcus* spp., *Clostridium* spp., and *Fusobacterium* spp.<sup>12,13</sup> Other anaerobic organisms with known intrinsic resistance include *Fusobacterium canifelinum*, which is intrinsically resistant to quinolones. Penicillin resistance can be common but is not predictable among these non-*Bacteroides* genera. To date, resistance to approved agents for *Clostridioides* (formerly *Clostridium*) *difficile* is rare; however, antimicrobial susceptibility testing (AST) is not often used in the clinical setting, because results do not imply efficacy for treating intraluminal infections.<sup>10</sup> For *C. difficile*, AST and typing may be useful for epidemiological purposes.<sup>14-16</sup> Refer to CLSI document M100<sup>1</sup> for a current antibiogram representing a four-year average from several laboratories.

Antimicrobial agent resistance among anaerobes correlates with the discovery and characterization of multiple, transferable resistance determinants corresponding to respective resistance phenotype(s),<sup>17</sup> and horizontal gene transfer is considered a major cause of rapid spread of resistance.<sup>18-20</sup> *Bacteroides fragilis*, in particular, is known as a reservoir for antimicrobial resistance determinants.<sup>16</sup> In addition, heavy use of some antimicrobial agents may result in the selection for and transfer of resistance determinants.<sup>17</sup> An important question is whether the observed antimicrobial agent resistance correlates with a poor clinical outcome. Factors limiting the ability to answer this question include:

- Nature of the infection (mixed aerobes and anaerobes)
- Lack of anaerobe identifications
- Lack of clinical data
- Use of inaccurate or modified susceptibility testing methods
- Effects of surgical drainage or debridement

However, studies on *Bacteroides* spp. bacteremia clearly demonstrate increased mortality and microbiological persistence for patients receiving ineffective therapy compared with those receiving effective therapy.<sup>21-24</sup> Furthermore, reports indicate that the incidence of anaerobic bacteremia is increasing.<sup>25,26</sup> The recent and varied trends in antimicrobial agent resistance, the spread of resistance genes, and the potential for poor clinical outcomes when using an ineffective antimicrobial agent indicate the need for increased AST on anaerobic organisms. The Working Group on Antimicrobial Susceptibility Testing of Anaerobic Bacteria has carefully considered these significant observations and has endeavored to develop reliable and reproducible methods for use in determining the antimicrobial susceptibility of these important pathogens. M11 includes guidance on:

- Number and species of organisms to test
- Testing frequency
- Selection of appropriate antimicrobial agents (see M100<sup>1</sup> Table 1C)

For the most current breakpoints, interpretive categories, and QC recommendations, refer to M100<sup>1</sup> Table 2J. Color plates illustrating both agar and broth microdilution end-point determinations are also included in M11 (see Subchapters 3.5.6 and 3.6.5). After rigorously evaluating and comparing methods, the working group is confident that AST can be reliably performed by the medical laboratory or performed at a referral laboratory using these or other comparable methods. Thus, in certain clinical situations, AST of anaerobic

isolates is recommended. At a minimum, AST for surveillance purposes should be strongly considered when expertise is available, or the isolate should be sent to a referral laboratory.

Because of standardization and correlation studies, agar dilution or broth microdilution are recommended for testing.<sup>27,28</sup> Although broth microdilution is used extensively for aerobic bacteria, limitations for anaerobic bacteria include lack of growth or poor growth of many species.<sup>29</sup> Because of poor strain growth due at least in part to excessive exposure to oxygen during set-up procedures, testing more fastidious anaerobes by this method provides inconsistent and unreliable results. Therefore, broth microdilution is recommended only for testing *Bacteroides* spp. and *Parabacteroides* spp. organisms.

The broth microdilution method has been evaluated with bacterial species other than *Bacteroides* spp. and *Parabacteroides* spp. Several requests for data comparing broth microdilution and agar dilution methods have been made. Based on data available at the time of this standard's publication, variable correlation was found between agar dilution MIC values and broth microdilution MIC values for *Clostridium* spp.<sup>30</sup> and gram-negative anaerobes other than *Bacteroides* spp. and *Parabacteroides* spp. Therefore, the recommendation for using the broth microdilution method for *Bacteroides* spp. and *Parabacteroides* spp. only is retained.<sup>30</sup>

MIC variability with some agents has been reported with *Eggerthella lenta* (*E. lentum*) ATCC<sup>®</sup> 43055. Therefore, QC ranges may not have been established for all antimicrobial agents with this organism. If MIC result variability is documented in early drug development studies (see CLSI document M23<sup>31</sup> QC tier 1<sup>a</sup>), this organism does not need to be included in CLSI document M23<sup>31</sup> QC tier 2 studies. Because of the problems associated with *E. lenta* ATCC<sup>®</sup> 43055, another QC organism has been established for testing agents active against gram-positive anaerobes. *C. difficile* ATCC<sup>®</sup> 700057 is a nontoxigenic strain, and QC values for relevant drugs are included in M100<sup>1</sup> Tables 5D and 5E. Furthermore, some QC ranges for the broth microdilution method were established before the method was restricted to *Bacteroides* spp. and *Parabacteroides* spp., and the QC ranges established following CLSI document M23<sup>31</sup> guidelines are still valid. Therefore, for historical and/or reference purposes, these ranges are still available in M100<sup>1</sup> Table 5E.

It is expected that new studies using the methods recommended in this edition will result in greater testing consistency and will serve as the reference standard for all future comparisons and clinical studies.

## Overview of Changes

This standard replaces the previous edition of the approved standard, M11-A8, published in 2012. Several changes were made in this edition, including:

- **General:**
  - Reorganized to fit the CLSI quality management system and path of workflow format
  - Made minor text revisions throughout for improved clarity and consistency with other CLSI documents
  - To align with the International Organization for Standardization, changed the name of the inoculum preparation method from growth method to broth culture method and changed direct colony suspension to colony suspension
  - Updated nomenclature for *Bacteroides fragilis* to *Bacteroides* spp. and *Parabacteroides* spp. per current standards

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<sup>a</sup> When referring to tier 1, 2, or 3 QC studies, the guidelines from CLSI document M23<sup>31</sup> are implied.

- **Subchapter 1.4, Terminology:**
  - Added definitions for epidemiological cutoff value, quality assurance, and saline
  - Revised definitions for breakpoint, interpretive category, and quality control for consistency with other CLSI documents
  - Added new abbreviations for consistency with other CLSI documents
- **Chapter 3, Antimicrobial Susceptibility Testing Process for Anaerobic Organisms:**
  - Added an anaerobic susceptibility testing process overview flow chart
- **Appendixes and tables:**
  - Reformatted Appendixes A, B, and C for improved clarity and workflow
  - Deleted Appendix D (Cumulative Antimicrobial Susceptibility Report for Anaerobic Organisms) and moved it to M100<sup>1</sup>
  - Deleted Table 1 (Suggested Groupings of Antimicrobial Agents Approved by the US Food and Drug Administration for Clinical Use That Should Be Considered for Testing and Reporting on Anaerobic Organisms by Microbiology Laboratories in the United States) and moved it to M100<sup>1</sup>

**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

Agar dilution, anaerobic bacteria, antimicrobial susceptibility, broth microdilution, minimal inhibitory concentration

**It is important for users of M11 to recognize that commercial susceptibility testing devices are not covered in this standard. The methods described herein are generic reference procedures that can be used for routine susceptibility testing by medical laboratories or that can be used by medical laboratories to evaluate commercial devices for possible routine use. Results generated by the CLSI reference methods are used by regulatory organizations to evaluate the performance of commercial systems before clearance is given. Regulatory agency clearance indicates the agency concludes that commercial devices provide susceptibility results that are substantially equivalent to results generated using the CLSI reference methods for the organisms and antimicrobial agents described in the manufacturer's approved package insert. Some laboratories may find that a commercial broth microdilution, agar gradient diffusion, colorimetric, turbidimetric, fluorometric, or other method is suitable for selective or routine use.**

## **Subcommittee on Antimicrobial Susceptibility Testing Mission Statement**

The 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 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 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 quality control 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.

# Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria

## Chapter 1: Introduction

This chapter includes:

- Standard's scope and applicable exclusions
- Background information pertinent to the standard's content
- Standard precautions information
- "Note on Terminology" that highlights particular use and/or variation in use of terms and/or definitions
- Terms and definitions used in the standard
- Abbreviations and acronyms used in the standard

### 1.1 Scope

This standard describes the CLSI antimicrobial susceptibility testing (AST) reference agar dilution method as well as the alternative broth microdilution method for *Bacteroides* spp. and *Parabacteroides* spp. organisms used to determine *in vitro* susceptibility to antimicrobial agents of bacteria that grow anaerobically. A method for  $\beta$ -lactamase testing on anaerobic bacteria is also described. This standard includes:

- Preparation of broth and agar dilution tests
- Testing conditions (including inoculum preparation and standardization, incubation time, and incubation temperature)
- Reporting minimal inhibitory concentration (MIC) results
- QC procedures
- Limitations of the dilution test methods
- A step-by-step guide to AST (see Appendix A)

To assist the medical laboratory, suggestions are provided for selecting antimicrobial agents for routine testing and reporting.

The disk diffusion method has not been standardized for use with anaerobic organisms and is not included. Methods for culturing and identifying anaerobic bacteria are not discussed. See CLSI document M56<sup>32</sup> for information on methods for culturing and identifying anaerobic bacteria. Methods for AST of aerobic bacterial species are also not discussed. 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