CLINICAL AND LABORATORY STANDARDS INSTITUTE®

9th Edition

M11

Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria

currently in preview, click buy full versi

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.

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

currently in preview, click buy full versi

M11, 9th ed.
October 2018
Replaces M11-A8

Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria

Darcie E. Carpenter, PhD, CIC, CEM
Karen (Kitty) Anderson
Diane M. Citron, BS
JoAnn L. Dzink-Fox, PhD, MS, MT(ASCP)
Meredith Hackel, PhD
Stephen G. Jenkins, PhD, D(ABMM), F(AAM)
Cindy Knapp, MS, BS, MT(ASCP)
Laura Koeth, MT(ASCP)
Audrey N. Schuetz, MD, MPH, D(ABMM)
Hannah Wexler, PhD

Currently in preview, click buy full version

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[1] tables, includes the most current information for drug selection, interpretation, QC, and antimicrobial 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.[1]

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.

The Clinical and Laboratory Standards Institute consensus process, which is the mechanism for moving a document through two or more levels of review by the health care community, is an ongoing process. Users should expect revised editions of any given document. Because rapid changes in technology may affect the procedures, methods, and protocols in a standard or guideline, users should replace outdated editions with the current editions of CLSI documents. Current editions are listed in the CLSI catalog and posted on our website at www.clsi.org. If you or your organization is not a member and would like to become one, or to request a copy of the catalog, contact us at: Telephone: +1.610.688.0100; Fax: +1.610.688.0700; E-Mail: customerservice@clsi.org; Website: www.clsi.org.

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

Copyright ©2018 Clinical and Laboratory Standards Institute. Except as stated below, any reproduction of content from a CLSI copyrighted standard, guideline, derivative product, or other material requires express written consent from CLSI. All rights reserved. Interested parties may send permission requests to permissions@clsi.org.

CLSI hereby grants permission to each individual member or purchaser to make a single reproduction of this publication for use in its laboratory procedures manual at a single site. To request permission to use this publication in any other manner, e-mail permissions@clsi.org.

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

currently in preview, click buy full versi

Committee Membership

Consensus Council

Dennis J. Ernst, MT(ASCP), NCPT(NCCT)
Chairholder
Center for Phlebotomy Education
USA

Mary Lou Gantzer, PhD, FACB
Vice-Chairholder
USA

J. Rex Astles, PhD, FACB, DABCC
Centers for Disease Control and Prevention
USA

Lucia M. Berte, MA, MT(ASCP)SBB, DLM, CQA(ASQ)CMQ/OE
Laboratories Made Better!
USA

Karen W. Dyer, MT(ASCP), DLM
Centers for Medicare & Medicaid Services
USA

Thomas R. Fritsche, MD, PhD, FCAP, FIDSA
Marshfield Clinic
USA

Loralie J. Langman, PhD, DABCC, FACB, F-ABFT
Mayo Clinic
USA

James R. Petisce, PhD
BD Diagnostic Systems
USA

Andrew Quintenz
Bio-Rad Laboratories, Inc.
USA

Robert Rej, PhD
New York State Department of Health – Wadsworth Center
USA

Zivana Tezak, PhD
FDA Center for Devices and Radiological Health
USA

Subcommittee on Antimicrobial Susceptibility Testing

Melvin P. Weinstein, MD
Chairholder
Rutgers Robert Wood Johnson Medical School
USA

Jean B. Patel, PhD, D(ABMM)
Vice-Chairholder
Centers for Disease Control and Prevention
USA

Sharon K. Cullen, BS, RAC
Beckman Coulter, Inc. Microbiology Business
USA

Marcelo F. Galas
Pan American Health Organization
USA

Howard Gold, MD, FIDS
Harvard Medical Faculty Physicians, BIDMC
USA

Romney M. Humphries, PhD, D(ABMM)
Accelerate Diagnostics
USA

Thomas J. Kirn, MD, PhD
Rutgers Robert Wood Johnson Medical School
USA

James S. Lewis, PharmD
Oregon Health and Science University
USA

Brandi Limbago, PhD
Centers for Disease Control and Prevention
USA

Amy J. Mathers, MD, D(ABMM)
University of Virginia Medical Center
USA

Tony Mazzulli, MD, FACP, FRCP(C)
Mount Sinai Hospital
Canada

Sandra S. Richter, MD, D(ABMM), FCAP, FIDSA
Cleveland Clinic
USA

Michael Satlin, MD
New York Presbyterian Hospital
USA

Audrey N. Schuetz, MD, MPH, D(ABMM)
Mayo Clinic
USA

Pranita D. Tamma, MD
Johns Hopkins University School of Medicine
USA

currently in preview, click

Working Group on Antimicrobial Susceptibility Testing of Anaerobic Bacteria

Darcie E. Carpenter, PhD, CIC, CEM
Chairholder
Beckman Coulter, Inc.
USA

Karen (Kitty)Anderson
Centers for Disease Control and Prevention
USA

Diane M. Citron, BS
R.M. Alden Research Laboratory
USA

JoAnn L. Dzink-Fox, PhD, MS, MT(ASCP)
Novartis Institutes for Biomedical Research
USA

Meredith Hackel, PhD
International Health Management Associates, Inc.
USA

Stephen G. Jenkins, PhD, D(ABMM), F(AAM)
Weill Cornell Medicine
USA

Cynthia C. Knapp, MS, BS, MT(ASCP)
Thermo Fisher Scientific
USA

Laura M. Koeth, MT(ASCP)
Laboratory Specialists, Inc.
USA

Audrey N. Schuetz, MD, MPH, D(ABMM)
Mayo Clinic
USA

Hannah Wexler, PhD
UCLA School of Medicine
USA

Staff

Clinical and Laboratory Standards Institute
USA

Marcy L. Hackenbrack, MCM, M(ASCP)
Project Manager

Megan L. Tertel, MA, ELS
Editorial Manager

Catherine E.M. Jenkins
Editor

Kristy L. Leirer, MS
Editor

Laura Martin
Editor

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.

Expert Panel on Microbiology

Richard B. Thomson, Jr., PhD, D(ABMM), FAAM
Chairholder
Evanston Hospital, NorthShore University HealthSystem
USA

Jean B. Patel
Vice-Chairholder
Centers for Disease Control and Prevention
USA

Kevin Alby, PhD, D(ABMM)
University of Pennsylvania Health System
USA

Lynette M. Berkeley, PhD, MT(ASCP)
FDA Center for Drug Evaluation and Research

Carey-Ann Burnham, PhD, D(ABMM)
Washington University School of Medicine
USA

Melissa Culbreath, PhD, D(ABMM)
TriCore Reference Laboratories
USA

German Esparza, BSc
Proasecal SAS
Colombia

Margie Morgan, PhD, D(ABMM)
Cedars-Sinai Medical Center
USA

Mark G. Papich, DVM, MS
College of Veterinary Medicine, North Carolina State University
USA

David H. Pincus, MS, RM/SM(NRCM), SM(ASCP)
bioMérieux, Inc.
USA

Audrey N. Schuetz, MD, MPH, D(ABMM)
Mayo Clinic
USA

Ribhi M. Shawar, PhD, D(ABMM)
FDA Center for Devices and Radiological Health
USA

currently in preview, click buy full versi

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:

George M. Eliopolous, MD
Beth Israel Deaconess Medical Center
USA

Robin Patel, MD
Mayo Clinic
USA

Barbara L. Zimmer, PhD
Beckman Coulter – West Sacramento
USA

currently in preview, click buy full versi

Contents

Currently in preview, click

Foreword

High-level antimicrobial agent resistance among anaerobic organisms is continually reported.[2-6] Resistance rates vary among species and from institution to institution.[5] Even within the same species, minimal inhibitory concentrations (MICs) to particular agents may vary significantly.[7] 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.[6,8-11]

Significant resistance rates are also identified in many non-*Bacteroides* anaerobe species, including *Prevotella* spp., *Peptostreptococcus* spp., *Clostridium* spp., and *Fusobacterium* spp.[12,13] 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.[10] For *C. difficile*, AST and typing may be useful for epidemiological purposes.[14-16] Refer to CLSI document M100[1] 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),[17] and horizontal gene transfer is considered a major cause of rapid spread of resistance.[18-20] *Bacteroides fragilis,* in particular, is known as a reservoir for antimicrobial resistance determinants.[16] In addition, heavy use of some antimicrobial agents may result in the selection for and transfer of resistance determinants.[17] 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.[21-24] Furthermore, reports indicate that the incidence of anaerobic bacteremia is increasing.[25,26] 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 of 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[1] Table 1C)

For the most current breakpoints, interpretive categories, and QC recommendations, refer to M100[1] 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

currently in preview, click buy full versi

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.[27,28] Although broth microdilution is used extensively for aerobic bacteria, limitations for anaerobic bacteria include lack of growth or poor growth of many species.[29] 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.[30] 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.[30]

MIC variability with some agents has been reported with *Eggerthella lenta* (*E. lenta*) ATCC® 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[31] QC tier 1[a]), this organism does not need to be included in CLSI document M23[31] QC tier 2 studies. Because of the problems associated with *E. lenta* ATCC® 43055, another QC organism has been established for testing agents active against gram-positive anaerobes. *C. difficile* ATCC® 700057 is a nontoxigenic strain, and QC values for relevant drugs are included in M100[1] 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[31] guidelines are still valid. Therefore, for historical and/or reference purposes, these ranges are still available in M100[1] 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

[a] When referring to tier 1, 2, or 3 QC studies, the guidelines from CLSI document M23[31] are implied.

currently in preview, click buy full versi

- **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[1]
  - 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[1]

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 β-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[32] 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

©*Clinical and Laboratory Standards Institute. All rights reserved.*