

M11-A7
Vol. 27 No. 2
Replaces M11-A6
Vol. 24 No. 2

Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard—Seventh Edition

This standard provides reference methods for the determination of minimal inhibitory concentrations (MICs) of anaerobic bacteria by agar dilution and broth microdilution.

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



M11-A7
ISBN 1-56238-626-3
ISSN 0273-3099

Volume 27 Number 2

Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard—Seventh Edition

David W. Hecht, MD
Diane M. Citron M(ASCP)
Mike Cox
Nilda Jacobus
Stephen G. Jenkins, PhD

Andrew Onderdonk, PhD
Darcie Roe-Carpenter, PhD
Jon E. Rosenblatt, MD
Hannah M. Wexler, PhD

Abstract

Susceptibility testing is indicated for any organism that contributes to an infectious process warranting antimicrobial chemotherapy if its susceptibility cannot reliably be predicted from existing antibiograms. Antimicrobial resistance patterns for many anaerobic bacteria have changed significantly over the last 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.

Two endpoint-determining susceptibility testing methods for anaerobic bacteria are described in this standard. The agar dilution method (Wadsworth) remains the reference standard, and is well suited for surveillance testing and research. It is also the standard to which other methods are compared. Broth microdilution is well suited for the clinical laboratory, but is currently limited to testing of *Bacteroides fragilis* group organisms and selected antibiotics. Quality control criteria for each procedure are also described.

The tabular information presented represents the most current information for drug selection, interpretation, and quality control. Users should replace tables published in earlier standards with these new tables. (Changes in the tables since the most recent edition appear in boldface type). 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 as informational supplements.

Clinical and Laboratory Standards Institute (CLSI). *Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard—Seventh Edition*. CLSI document M11-A7 (ISBN 1-56238-626-3). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2007.

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Foreword

Antibiotic resistance among anaerobic organisms has increased significantly in recent years.¹⁻³ Resistance rates vary among species and also from hospital to hospital. Even within the same species, MICs to particular agents may vary significantly.^{2,4,5} For example, among the various members of the *Bacteroides fragilis* group reported resistance rates include clindamycin (15 to 44%), cefotetan (13 to 94%), and cefoxitin (3.5 to 41.5%). Resistance to even the most active drugs such as imipenem, piperacillin-tazobactam, ampicillin-sulbactam, and metronidazole is found in occasional strains.⁶ Frequently, the other species in the *B. fragilis* group are more resistant than *B. fragilis* to many antibiotics. Variations in susceptibility are both species- and hospital-dependent. Virtually all *Bacteroides fragilis* group spp. are resistant to penicillin. Among non-*Bacteroides* anaerobes, significant resistance is also identified in many species, including *Prevotella* spp. (penicillin, 50%; clindamycin, 17%; piperacillin, 11%; cefotetan, 6%), *Peptostreptococcus* spp. (clindamycin, 16%), *Clostridium* spp. (clindamycin, 11%; cefotetan, 12%; cefoxitin 14%), and *Fusobacterium* spp. (ceftizoxime 18%).^{1,2,7} Other anaerobic organisms with known intrinsic resistance include *Sutterella wadsworthensis* and *Bilophila wadsworthia*. Among these non-*Bacteroides* genera, penicillin resistance can be common but is not predictable. Current antibiograms of anaerobic bacteria have been summarized and reviewed recently.^{3,8} The increasing and prevalent antibiotic resistance among anaerobic organisms is correlated with the discovery and characterization of multiple, transferable resistance determinants corresponding to their respective resistance phenotype(s).³ In addition, heavy use of some antibiotics may result in the selection for, and transfer of, these resistance determinants.¹

An important question is whether the observed antibiotic resistance correlates with poor clinical outcome. Until recently, studies demonstrating such a correlation were few and retrospective in nature.⁹ Factors limiting these studies include the nature of the infection (mixed aerobes and anaerobes), the lack of identification of anaerobes, a lack of clinical data, the use of inaccurate or modified susceptibility testing methods, and the effects of surgical drainage or debridement. However, recent studies of *Bacteroides* bacteremia clearly demonstrate increased mortality and microbiological persistence for patients receiving ineffective therapy compared with those receiving effective therapy.¹⁰⁻¹³

The recent and varied trends in antibiotic resistance, the spread of resistance genes, and the potential for poor clinical outcomes when using an ineffective antibiotic argue strongly for more susceptibility testing of anaerobic organisms. The anaerobe working group has carefully considered these significant observations, and has endeavored to develop reliable and reproducible methods that can be used to determine the susceptibility of these important pathogens. M11-A7 contains a step-by-step guide to susceptibility testing (Appendix B), including guidance on the number and species of organisms to test, how often to test, and selection of appropriate antibiotics (Table 1). Color plates illustrating both agar and broth microdilution endpoint determinations are also included in this edition (Figures 2 and 3). This protocol serves as a standard to which other methods may be compared.

As a result of rigorous evaluation and comparison among these methods, the working group is confident that susceptibility testing can be reliably performed by the clinical laboratory, or performed at a reference laboratory using these or other comparable methods. Thus, the anaerobe working group recommends (in certain clinical situations) susceptibility testing of anaerobic isolates. At a minimum, susceptibility testing for surveillance purposes should be strongly considered utilizing these or validated equivalent methods when expertise is available, or the isolate should be sent to a reference laboratory.

As a result of the standardization and correlation studies performed by the working group, either of two methods is recommended for testing—agar dilution or broth microdilution.^{14,15} Recognizing that while broth microdilution is utilized extensively, limitations exist that include lack of growth or poor growth of many anaerobic species.¹⁶ Testing other, more fastidious anaerobes by this method gives inconsistent and unreliable results because of poor growth of the strains, due, at least in part, to excessive exposure to oxygen during set-up procedures. Therefore, this method is only recommended by CLSI for *B. fragilis*

group organisms, although there are commercial broth microdilution panels that are FDA-approved for testing all anaerobes, and may work satisfactorily for certain non-*B. fragilis* group species.

In recognition of the problems associated with *Eubacterium lentum* ATCC® 43055, the working group has established a new QC strain to be used for testing agents active against gram-positive anaerobes. *Clostridium difficile* ATCC® 700057 is a nontoxigenic strain, and agar dilution QC values for 23 drugs are included in Table 5. The working group plans to establish QC ranges for additional antimicrobials for both agar and broth microdilution testing.

The working group expects that new studies using the methods recommended in this edition will result in greater consistency in testing, and will serve as the gold standard for all future comparisons and clinical studies. Clinical laboratories may find a commercial broth microdilution or agar gradient method^{17,18} convenient for routine testing of patients' isolates. The manufacturer's directions should be followed carefully when using the device. Some clinical laboratories may choose to perform other, simpler methods, such as a concentration gradient agar diffusion method. Should alternative methods be used, laboratories should ensure that the QC values are within acceptable ranges.

David W. Hecht, MD

Chairholder, Working Group on Susceptibility Testing of Anaerobic Bacteria

Key Words

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

It is important for users of M11-A7 to recognize that commercial susceptibility testing devices are not addressed in this standard. The methods described herein are generic reference procedures that can be used for routine susceptibility testing by clinical laboratories, or that can be used by clinical laboratories to evaluate commercial devices for possible routine use. Results generated by the CLSI reference methods are used by the United States Food and Drug Administration to evaluate the performance of commercial systems before clearance is given for marketing in the United States. Clearance by the FDA indicates that 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 could find that a commercial dilution, antibiotic gradient, colorimetric, turbidimetric, fluorometric, or other method is suitable for selective or routine use.

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1 Scope

The methods described in this document are intended for testing commonly isolated anaerobic bacteria. The agar dilution method may be used to test a wide variety of anaerobic organisms. Currently the broth microdilution method using the recommended medium is only suggested for testing organisms from the *Bacteroides fragilis* group. When other applications have been validated, they will be published in annual updates of CLSI document M100—*Performance Standards for Antimicrobial Susceptibility Testing*.

2 Introduction

This document describes the CLSI reference agar dilution method, the alternative broth microdilution method for *B. fragilis* group organisms, and a method for β -lactamase testing for anaerobic bacteria. The agar dilution method has been studied extensively in collaborative multicenter trials and is the recommended reference method for all anaerobic organisms. The broth microdilution procedure is useful as a more “user-friendly” method that allows testing of multiple antimicrobial agents on one microdilution tray. However, recent multilaboratory collaborative studies comparing broth microdilution to agar dilution using the medium recommended in this edition limit its current application to members of the *B. fragilis* group for some antibiotics (see Foreword). For those agents tested to date (Table 2), the methods are considered equivalent. To perform the tests, twofold dilution series of antimicrobial agents are prepared in agar and added to Petri plates or prepared in broth and added to wells of a microdilution plate. A standardized suspension of the test organism is then inoculated onto each agar surface or into each well. After incubation for 42 to 48 hours, growth on each plate or in each well is examined and the minimal inhibitory concentration (MIC) is determined. Careful adherence to the methodology described here is essential to achieving reproducible (interlaboratory and intralaboratory) results.

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 infectious agents and thus are more comprehensive than universal precautions, which are intended to apply only to transmission of blood-borne pathogens. Standard and universal precaution guidelines are available from the U.S. Centers for Disease Control and Prevention (Garner JS, Hospital Infection Control Practices Advisory Committee. Guideline for isolation precautions in hospitals. *Infect Control Hosp Epidemiol.* 1996;17(1):53-80). For specific precautions for preventing the laboratory transmission of all infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all infectious disease, refer to the most current edition of CLSI document M29—*Protection of Laboratory Workers From Occupationally Acquired Infections*.

4 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.

Related CLSI/NCCLS Publications*

- M6-A2** **Protocols for Evaluating Dehydrated Mueller-Hinton Agar; Approved Standard—Second Edition (2005).** This standard describes three protocols for the evaluation of dehydrated Mueller-Hinton agar in the disk
- M7-A7** **Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Seventh Edition (2006).** This document addresses reference methods for the determination of minimal inhibitory concentrations (MICs) of aerobic bacteria by broth macrodilution, broth microdilution, and agar dilution.
- M23-A2** **Development of *In Vitro* Susceptibility Testing Criteria and Quality Control Parameters; Approved Guideline—Second Edition (2001).** This document addresses the required and recommended data needed for the selection of appropriate interpretive standards and quality control guidelines for antimicrobial agents.
- M29-A3** **Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Third Edition (2005).** Based on U.S. 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.
- M39-A2** **Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data; Approved Guideline—Second Edition (2002).** This document describes methods for recording and analysis of antimicrobial susceptibility test data, consisting of cumulative and ongoing summaries of susceptibility patterns of clinically significant microorganisms.

* Proposed-level documents are being advanced through the Clinical and Laboratory Standards Institute consensus process; therefore, readers should refer to the most recent editions.