M11-A8

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

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

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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 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 end-point–determining susceptibility testing methods for anaerobic bacteria are described in Clinical and Laboratory Standards Institute document M11-A8—Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard—Eighth Edition. 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 \textit{Bacteroides fragilis} group organisms and selected antimicrobial agents. QC criteria for each procedure are also described.

The tables in CLSI document M100,\textsuperscript{1} when used in conjunction with this standard, represent the most current information for drug selection, interpretation, QC, and antibiogram reports using the procedures standardized in M11. Users should replace tables published in earlier standards with these new tables. (Changes in the tables since the previous edition appear in boldface type). When new problems are recognized, or improvements in these criteria are made, changes will be incorporated into future editions of this standard and also distributed as informational supplements.

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Foreword

High levels of antimicrobial agent resistance among anaerobic organisms are continually reported. Resistance rates vary among species and also from hospital to hospital. Even within the same species, minimal inhibitory concentrations (MICs) to particular agents may vary significantly. Among *Bacteroides* species, resistance to some commonly used antimicrobial agents (eg, clindamycin, moxifloxacin, piperacillin) can be very high; significant variability can occur among isolates from hospitalized patients in multiple institutions even in the same geographic region. Resistance has even been reported among the most active drugs, such as imipenem, piperacillin-tazobactam, tigecycline, ampicillin-sulbactam, and metronidazole. Significant rates of resistance are also identified in many non-*Bacteroides* anaerobe species, including *Prevotella* spp., *Peptostreptococcus* spp., *Clostridium* spp., and *Fusobacterium* spp. Other anaerobic organisms with known intrinsic resistance include *Sutterella wadsworthensis* and *Bilophila wadsworthia*. Penicillin resistance can be common but is not predictable among these non-*Bacteroides* genera. To date, resistance to approved agents for *Clostridium difficile* is rare; however, susceptibility testing has been useful to correlate high MICs to some antimicrobial agents and epidemiology of typed isolates. A current antibiogram representing a three-year average from one or more laboratories appears in Appendixes D and E. Antimicrobial agent resistance among anaerobic organisms is correlated with the discovery and characterization of multiple, transferable resistance determinants corresponding to their respective resistance phenotype(s). In addition, heavy use of some antimicrobial agents may result in the selection for, and transfer of, these resistance determinants.

An important question is whether the observed antimicrobial agent resistance correlates with a poor clinical outcome. Factors that limit the ability to answer this question include the nature of the infection (mixed aerobes and anaerobes), the lack of identification of anaerobes, the lack of clinical data, the use of inaccurate or modified susceptibility testing methods, and the effects of surgical drainage or debridement. However, studies of *Bacteroides* bacteremia clearly demonstrate increased mortality and microbiological persistence for patients receiving ineffective therapy compared with those receiving effective therapy. Furthermore, recent reports indicate that the incidence of anaerobic bacteremia is increasing.

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 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 contains a step-by-step guide to susceptibility testing (see Appendix B), including guidance on the number and species of organisms to test, frequency of testing, and selection of appropriate antimicrobial agents (see Table 1). For the most current interpretive breakpoints and QC recommendations refer to Tables 2J, 4D, and 4E in CLSI document M100. Color plates illustrating both agar and broth microdilution end-point determinations are also included in this edition (see 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 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. Although broth microdilution is used extensively, limitations exist that include lack of growth or poor growth of many anaerobic species. 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 recommended by CLSI only for *B. fragilis* group organisms. There are some commercial broth microdilution panels that may work satisfactorily for certain non-*B. fragilis* species. However, for a panel to be US Food and Drug Administration (FDA) Center for Devices and Radiological Health–cleared, all drugs on the panel must be FDA Center for Drug Evaluation and Research–approved. If even one drug does not have an FDA drug label, the panel becomes “research use only” and the user must do his or her own validation. At time of publication, there is one FDA-approved broth microdilution panel for anaerobic susceptibility testing.

In recognition of the problems associated with *Eggerthella lenta* ATCC® 43055, the working group has established a new QC organism for use in testing agents active against gram-positive anaerobes. *C. difficile* ATCC® 700057 is a nontoxigenic strain, and agar dilution QC values for 23 drugs are included in Table 4D in CLSI document M100.¹

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 method²²,²³ convenient for routine testing of patients’ isolates. The manufacturer’s directions should be followed carefully when using the device, and 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

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¹ ATCC® is a registered trademark of the American Type Culture Collection.

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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 and guidelines 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 interpretive criteria for the results of standard antimicrobial susceptibility tests.
- Provide suggestions for testing and reporting strategies that are clinically relevant and cost-effective.
- Continually refine standards and optimize the detection of emerging resistance mechanisms through the development of new or revised methods, interpretive criteria, 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 standards and guidelines. The values that guide this mission are quality, accuracy, fairness, timeliness, teamwork, consensus, and trust.
Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard—Eighth Edition

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 variety of anaerobic organisms. Currently, the broth microdilution method is suggested only for testing organisms from the *Bacteroides fragilis* group. If and when other applications are approved, they will be published in subsequent versions of M11 and in annual updates of CLSI document M100.¹

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 is the recommended reference method for all anaerobic organisms. The broth microdilution procedure is 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 antimicrobial agents (see Foreword). For those agents tested to date, the methods are considered equivalent. Briefly, the tests are performed by preparing twofold dilution series of antimicrobial agents in either agar plates or broth (added to wells of a microtiter plate). A standardized suspension of the test organism is then inoculated onto each agar surface or into each well. After incubation, the growth on each plate or in each well is examined and the minimal inhibitory concentration (MIC) is determined. Careful adherence to the methodology described herein 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 known 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 Centers for Disease Control and Prevention.² 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 M29.²⁵

4 Terminology

4.1 A Note on Terminology

CLSI, as a global leader in standardization, is firmly committed to achieving global harmonization wherever possible. Harmonization is a process of recognizing, understanding, and explaining differences while taking steps to achieve worldwide uniformity. CLSI recognizes that medical conventions in the global metrological community have evolved differently in the United States, Europe, and elsewhere; that these differences are reflected in CLSI, International Organization for Standardization (ISO), and European Committee for Standardization (CEN) documents; and that legally required use of terms, regional usage, and different consensus timelines are all important considerations in the harmonization
process. In light of this, CLSI’s consensus process for development and revision of standards and guidelines focuses on harmonization of terms to facilitate the global application of standards and guidelines.

In order to align the usage of terminology in this document with that of ISO, the term *accuracy*, in its metrological sense, refers to the closeness of the agreement between the result of a (single) measurement and a true value of a measurand, and comprises both random and systematic effects.

### 4.2 Definitions

**accuracy (measurement)** – closeness of agreement between a measured quantity value and a true quantity value of a measurand (JCGM 200:2008).

**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** – 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) **intermediate** – a category that includes isolates with antimicrobial agent minimal inhibitory concentrations (MICs) that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates. **NOTE:** The intermediate category implies clinical efficacy in body sites where the drugs are physiologically concentrated (eg, quinolones and β-lactams in urine) or when a higher than normal dosage of a drug can be used (eg, β-lactams). This category also includes a buffer zone, which should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations, especially for drugs with narrow pharmacotoxicity margins.

3) **resistant** – a category that implies that isolates are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules and/or that demonstrate MICs that fall in the range in which specific microbial resistance mechanisms (eg, β-lactamases) are likely, and clinical efficacy of the agent against the isolate has not been reliably shown in treatment studies.

4) **nonsusceptible** – a category used for isolates for which only a susceptible interpretive criterion has been designated because of the absence or rare occurrence of resistant strains. Isolates that have MICs above or zone diameters below the value indicated for the susceptible breakpoint should be reported as nonsusceptible; **NOTE 1:** An isolate that is interpreted as nonsusceptible does not necessarily mean that the isolate has a resistance mechanism. It is possible that isolates with MICs above the susceptible breakpoint that lack resistance mechanisms may be encountered within the wild-type distribution subsequent to the time the susceptible-only breakpoint is set; **NOTE 2:** For strains yielding results in the “nonsusceptible” category, organism identification and antimicrobial susceptibility test results should be confirmed.

**minimal inhibitory concentration (MIC)** – the lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism in an agar or broth dilution susceptibility test; **NOTE:** See Section 12.4, Reading Agar Dilution Plates, and Section 13.5, Reading Broth Microdilution End Points.

**quality control (QC)** – the operational techniques and activities that are used to fulfill requirements for quality (modified from ISO 9000); **NOTE:** A system for ensuring maintenance of proper standards by periodic inspection of the results and the operational techniques that are used to ensure accuracy and reproducibility.
The Quality Management System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management system 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 quality management system 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 as follows:

- Organization
- Personnel
- Process Management
- Nonconforming Event Management
- Customer Focus
- Purchasing and Inventory
- Documents and Records
- Assessments
- Facilities and Safety
- Equipment
- Information Management
- Continual Improvement

M11-A8 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.

M11-A8 addresses the clinical laboratory path of workflow processes indicated by an “X.” For a description of the other document listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.
Related CLSI Reference Materials*

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-A3 Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Third Edition (2005). 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.


M100-S22 Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement (2012). This document provides updated tables for the Clinical and Laboratory Standards Institute antimicrobial susceptibility testing standards M02-A11 and M07-A9.

* CLSI documents are continually reviewed and revised through the CLSI consensus process; therefore, readers should refer to the most current editions.

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