Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Eighth Edition

This document addresses reference methods for the determination of minimal inhibitory concentrations (MICs) 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.
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Advancing Quality in Health Care Testing

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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 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.

A variety of laboratory methods can be used to measure the *in vitro* susceptibility of bacteria to antimicrobial agents. This document describes standard broth dilution (macrodilution and microdilution [the microdilution method described in M07 is the same methodology outlined in ISO 20776-1]) 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 tables) presented with this standard represents the most current information for drug selection, interpretation, and quality control using the procedures standardized in M07. These tables, as in previous years, have been updated and should replace tables published in earlier years. Changes in the tables since the previous edition (M100-S18) appear in boldface type and are also summarized in the front of the document.


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in technology may affect the procedures, methods, and protocols in a standard or guideline, users should replace outdated editions with the current editions of CLSI/NCCLS documents. Current editions are listed in the CLSI catalog and posted on our website at www.clsi.org. If your organization is not a member and would like to become one, and to request a copy of the catalog, contact us at: Telephone: 610.688.0100; Fax: 610.688.0700; E-Mail: customerservice@clsi.org; Website: www.clsi.org

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Foreword

In this 2009 revision of CLSI document M07, several sections have been added or revised as outlined in the Summary of Changes. The latest version of the M100 tables (M100-S19) published as an annual volume is made available with this document to ensure that users are aware of the latest subcommittee guidelines related to both methods and the tabular information normally presented in the annual tables. M100-S19 will be updated during subcommittee meetings in 2009 and published again as a separate document in January 2010.

Many other editorial and procedural changes in this edition of M07 resulted from meetings of the Subcommittee on Antimicrobial Susceptibility Testing since 2006. Specific changes for the M100 tables are summarized at the beginning of the M100-S19 document. The most important changes in the M07 document are summarized below.

It has been an honor to serve as Chairholder of the Subcommittee on Antimicrobial Susceptibility Testing during the last three years. Many members of the subcommittee, which now numbers more than 180 volunteers including members, advisors, and observers, have been indispensable in the preparation of these documents. In addition, I would like to thank the chairholders of the working groups of the Subcommittee on Antimicrobial Susceptibility Testing for their valuable contributions during the last three years. They include Jana Swenson (Text and Table Revision and Acinetobacter Working Groups); Frank Cockerill (Agents of Bioterrorism Working Group); Sharon Cullen and Steve Brown (Quality Control Working Group); Dwight Hardy (Stenotrophomonas and Burkholderia Working Group); George Eliopoulos (M23—Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters Working Group); John McGowan (Communications Working Group); Janet Hindler (M39—Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data Working Group); David Hecht (M11—Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria Working Group); Fred Tenover (Staphylococci Working Group); Mike Dudley (Enterobacteriaceae Working Group); Jim Jorgensen (M45—Methods for Antimicrobial Dilution and Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria Working Group); and Barth Reller (Table 1 Working Group).

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Summary of Major Changes in This Document

Summary of CLSI Processes for Establishing Interpretive Criteria and QC Ranges
Added information on the process utilized by the Subcommittee on Antimicrobial Susceptibility Testing and the data that are required to establish interpretive criteria, quality control parameters for updating this document.

Added URL for locating minutes from Subcommittee on Antimicrobial Susceptibility Testing meetings

CLSI Reference Methods vs Commercial Methods and CLSI vs FDA Breakpoints (interpretive criteria)
New heading for text box.

Section 4.1, Definitions
Added definitions for D-zone test, quality assurance (QA), nonsusceptible, and saline.

Section 4.2, Abbreviations/Acronyms
Added an Abbreviations/Acronyms section.

Section 6.2.2.5, Macrolides
Listed the subgroups of antimicrobials for the macrolide group.
Summary of Major Changes in This Document (Continued)

Section 6.2.2.7, Tetracyclines
Added information on tigecycline, a glycylcycline.

Moved instructions for media and reagent preparation to Appendix B, which include those from:
Section 8.1, Turbidity standard for inoculum preparation
Section 9.1.1, Mueller-Hinton agar
Section 10.1, Mueller-Hinton broth
Section 11.1, *Haemophilus* Test Medium (HTM)
Section 11.2, GC agar
Section 11.3, Mueller-Hinton agar supplemented with 5% sheep blood
Section 11.4, CAMHB with 2.5% to 5% lysed horse blood

Section 11.3, *Neisseria meningitidis*
Added cautionary statement for performing susceptibility testing in a biological safety cabinet.

Section 12.1.3.1, Methods for Detection of Reduced Susceptibility to Vancomycin
Added table summarizing the various methods to detect levels of vancomycin susceptibility in *S. aureus*.

Section 12.1.3.3, Heteroresistant Vancomycin-Intermediate *Staphylococcus aureus* (hVISA)
Added discussion of hVISA.

Section 12.1.5, Mupirocin Resistance
Added method for detecting and reporting high-level mupirocin resistance (ie, MICs ≥ 512 µg/mL) in *S. aureus*.

Section 12.3, β-Lactamase-Mediated Resistance in Gram-Negative Bacilli
Added table showing the molecular classification of β-lactamases and discussion of plasmid-encoded β-lactamases, *Klebsiella pneumoniae* carbapenemase (KPC) carbapenemases, AmpC β-lactamases, and metallo-β-lactamases.

Section 16.2, Quality Control Responsibilities
Added new section outlining the quality control responsibilities of both manufacturers and users.

Section 16.3, Selection of Quality Control Strains for Quality Control and Quality Assurance
Expanded section on using, selecting, and obtaining quality control strains and defined QC strain and supplemental QC strain.

Section 16.7.1, Daily Testing
Clarified consecutive results as consecutive test days.

Section 16.9.1, Out-of-Control Result Due to Identifiable Errors
Expanded on the possible causes for out-of-control results and strategy for corrective action.

Section 16.9.2, Out-of-Control Result With No Error Identified
Expanded on the possible causes for out-of-control results and strategy for corrective action.
Summary of Major Changes in This Document (Continued)

Section 16.12, Other Control Procedures
Added section outlining inoculum control and end-point interpretation control.

Appendix B, Preparation of Supplements, Media, and Reagents
Added new appendix listing media and reagent preparation instructions.

Appendix C, Conditions for Dilution Antimicrobial Susceptibility Tests
Added new appendix providing medium, incubation temperature, incubation time, and minimal quality control for organisms addressed in this document and listed in M100 Table 2 series.

Appendix D, Quality Control Strains for Antimicrobial Susceptibility Tests
New appendix providing quality control organism characteristics.

Appendix E, Quality Control Strain Maintenance
Added new appendix providing steps for quality control strain maintenance.
Summary of CLSI Processes for Establishing Interpretive Criteria and QC Ranges

The Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) is an international, voluntary, nonprofit, interdisciplinary, standards-developing, and educational organization accredited by the American National Standards Institute (ANSI), that develops and promotes use of consensus developed standards and guidelines within the health care community. These consensus standards and guidelines are developed to address critical areas of diagnostic testing and patient health care and are developed in an open and consensus seeking forum. CLSI is open to anyone, or any organization that has an interest in diagnostic testing and patient care. Information about CLSI is found at www.clsi.org.

The CLSI Subcommittee on Antimicrobial Susceptibility Testing reviews data from a variety of sources and studies (eg, in vitro, pharmacokinetics/pharmacodynamics, and clinical studies) to establish antimicrobial susceptibility test methods, interpretive criteria, and quality control (QC) parameters. The details of the data required to establish interpretive criteria, quality control parameters and how the data are to be presented for evaluation are described in CLSI document M23.²

Over time, a microorganism’s susceptibility to an antimicrobial agent may decrease, resulting in a lack of clinical efficacy and/or safety. In addition, microbiological methods and QC parameters may be refined to ensure more accurate and better performance of susceptibility test methods. Because of this, CLSI continually monitors and updates information in its documents. While CLSI standards and guidelines are developed using the most current information and thinking available at the time, the field of science and medicine is ever changing; therefore, standards and guidelines should be used in conjunction with clinical judgment, current knowledge, and clinically relevant laboratory test results to guide patient treatment.

Additional information, updates, and changes in this document are found in the meeting summary minutes of the Subcommittee on Antimicrobial Susceptibility Testing at www.clsi.org.
It is important for users of M02-A10 and M07-A8 to recognize that commercial susceptibility testing devices are not addressed in these standards. 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 US Food and Drug Administration (FDA) 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.

CLSI breakpoints may differ from those approved by various regulatory authorities for many reasons, including the following: different databases, differences in interpretation of data, differences in doses utilized in different parts of the world, and public health policies. Differences also exist because CLSI proactively evaluates the need for changing breakpoints. The reasons why breakpoints may change and the manner in which CLSI evaluates data and determines breakpoints are outlined in CLSI document M23—Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters.

Following a decision by CLSI to change an existing breakpoint, regulatory authorities may also review data in order to determine how changing breakpoints may affect the safety and effectiveness of the antimicrobial agent for the approved indications. If the regulatory authority changes breakpoints, commercial device manufacturers may have to conduct a clinical laboratory trial, submit the data to the regulatory authority, and await review and approval. For these reasons, a delay of more than the suggested CLSI “tentative” period of one year may be required if an interpretive breakpoint change is to be implemented by a device manufacturer. In the United States, laboratories that use Food and Drug Administration (FDA)-approved susceptibility testing devices are allowed to utilize existing FDA interpretive breakpoints. Either FDA or CLSI susceptibility interpretive breakpoints are acceptable to clinical laboratory accrediting bodies. Policies in other countries may vary.

Following discussions with appropriate stakeholders such as infectious disease practitioners and the pharmacy department, as well as the Pharmacy and Therapeutics and Infection Control committees of the medical staff, newly approved or revised breakpoints may be implemented by clinical laboratories. CLSI broth dilution and agar dilution test breakpoints may be implemented as soon as they are published in M100. If a device includes antimicrobial test concentrations sufficient to allow interpretation of susceptibility to an agent using the CLSI breakpoints, a laboratory could, after appropriate validation, choose to interpret and report results using CLSI breakpoints.
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, healthcare 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 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 detection of emerging resistance mechanisms through 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 guidelines. The values that guide this mission are quality, accuracy, fairness, timeliness, teamwork, consensus, and trust.

Key Words

Agar dilution, antimicrobial susceptibility, broth dilution, macrodilution, microdilution, minimal inhibitory concentration (MIC)
Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Eighth Edition

1 Scope

This document describes the standard broth (macrodilution and microdilution) and agar dilution methods used to determine the in vitro susceptibility of bacteria that grow aerobically. It addresses preparation of broth and agar dilution tests, testing conditions (including inoculum preparation and standardization, incubation time, and incubation temperature), reporting of minimal inhibitory concentration (MIC) results, quality control (QC) procedures, and limitations of the dilution test methods. To assist the clinical laboratory, suggestions are provided on the selection of antimicrobial agents for routine testing and reporting. Standards for testing the in vitro susceptibility of bacteria that grow aerobically utilizing the antimicrobial disk susceptibility testing method are found in CLSI document M02.3 Standards for testing the in vitro susceptibility of bacteria that grow anaerobically are found in CLSI document M11.4 Guidelines for standardized susceptibility testing of infrequently isolated or fastidious bacteria that are not included in CLSI documents M02,3 M07, or M114 are available in CLSI document M45.5

2 Introduction

Either broth or agar dilution methods may be used to measure quantitatively the in vitro activity of an antimicrobial agent against a given bacterial isolate. To perform the tests, a series of tubes or plates is prepared with a broth or agar medium to which various concentrations of the antimicrobial agents are added. The tubes or plates are then inoculated with a standardized suspension of the test organism. After incubation at 35 ± 2 °C, the tests are examined and the MIC is determined. The final result is significantly influenced by methodology, which must be carefully controlled if reproducible results (intralaboratory and interlaboratory) are to be achieved.

This document describes reference standard broth dilution (macrodilution and microdilution) and agar dilution methods. The basics of these methods are derived, in large part, from information generated by the International Collaborative Study.6 Although these methods are standard reference methods, some are sufficiently practical for routine use in both clinical laboratories and research laboratories.

Commercial systems based primarily, or in part, on certain of these methods are available and may provide essentially equivalent results to the CLSI methods described here. The US Food and Drug Administration (FDA) is responsible for the approval of commercial devices used in the United States. CLSI does not approve or endorse commercial products or devices.

The methods described in this document 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 Section 11 and M1007 Tables 2E through 2L. Methods for testing anaerobic bacteria are outlined in CLSI document M11.4 Methods for testing infrequently isolated or fastidious bacteria not included in M023 and M07 are found in CLSI document M45.5

This document, along with M100,7 describes methods, QC, and interpretive criteria 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 also distributed in M100,7 which provides annual informational supplements.
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 US Centers for Disease Control and Prevention (CDC). 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 CLSI document M29.

4 Terminology

4.1 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.

1) susceptible – a category that implies that isolates are inhibited by the usually achievable concentrations of antimicrobial agent when the recommended dosage is used for the site of infection.

2) intermediate – a category that includes isolates with antimicrobial agent 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 where specific microbial resistance mechanisms (eg, β-lactamases) are likely, and clinical efficacy of that agent against the isolate has not been reliably shown in treatment studies.

4) nonsusceptible – a category used for organisms that have only a susceptible interpretive category, but not intermediate or resistant interpretive categories (ie, susceptible-only interpretive category). A susceptible-only interpretive category may be applied to new antimicrobial agents for which no resistant isolates have been encountered at the time the initial interpretive criteria are determined. Isolates that test with an MIC above the susceptible interpretive breakpoint are designated as nonsusceptible. A designation of nonsusceptible does not necessarily mean that a resistance mechanism exists in the isolate. The MIC of the isolate in the nonsusceptible range may be within the previously recognized wild-type distribution of susceptibility results; however, there is limited clinical experience with these isolates in clinical trials.

D-zone test – a disk diffusion test using clindamycin and erythromycin disks placed in close proximity to detect the presence of inducible clindamycin resistance in staphylococci and streptococci.

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.
quality assurance (QA) – a part of quality management focused on providing confidence that quality requirements will be fulfilled (ISO 9000)\textsuperscript{12}; \textbf{NOTE}: The practice that encompasses all procedures and activities directed toward ensuring that a specified quality of product is achieved and maintained. In the testing environment, this includes monitoring all the raw materials, supplies, instruments, procedures, sample collection/transport/storage/processing, recordkeeping, calibrating and maintaining equipment, quality control, proficiency testing, training of personnel, and all else involved in the production of the data reported.

quality control (QC) – the operational techniques and activities that are used to fulfill requirements for quality (ISO 9000)\textsuperscript{12}; \textbf{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.

saline – a solution of 0.85\% to 0.9\% NaCl (w/v).

4.2 Abbreviations/Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC</td>
<td>American Type Culture Collection</td>
</tr>
<tr>
<td>BHI</td>
<td>Brain Heart Infusion</td>
</tr>
<tr>
<td>BLNAR</td>
<td>β-lactamase negative, ampicillin resistant</td>
</tr>
<tr>
<td>BSC</td>
<td>biological safety cabinet</td>
</tr>
<tr>
<td>BSL</td>
<td>Biosafety Level</td>
</tr>
<tr>
<td>CAMHB</td>
<td>cation-adjusted Mueller-Hinton broth</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
</tr>
<tr>
<td>CFU</td>
<td>colony forming units</td>
</tr>
<tr>
<td>CMRNG</td>
<td>chromosomally mediated penicillin-resistant \textit{Neisseria gonorrhoeae}</td>
</tr>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>ESBL</td>
<td>extended-spectrum β-lactamase</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration (USA)</td>
</tr>
<tr>
<td>HPLC</td>
<td>high-performance liquid chromatography</td>
</tr>
<tr>
<td>HTM</td>
<td>\textit{Haemophilus} Test Medium</td>
</tr>
<tr>
<td>hVISA</td>
<td>heteroresistant vancomycin-intermediate \textit{Staphylococcus aureus}</td>
</tr>
<tr>
<td>KPC</td>
<td>\textit{Klebsiella pneumoniae} carbapenemase</td>
</tr>
<tr>
<td>LHB</td>
<td>lysed horse blood</td>
</tr>
<tr>
<td>MDR</td>
<td>multidrug resistant</td>
</tr>
<tr>
<td>MHA</td>
<td>Mueller-Hinton agar</td>
</tr>
<tr>
<td>MHB</td>
<td>Mueller-Hinton broth</td>
</tr>
<tr>
<td>MIC</td>
<td>minimal inhibitory concentration</td>
</tr>
<tr>
<td>MLS\textsubscript{B}</td>
<td>macrolide, lincosamide, and type B streptogramin</td>
</tr>
<tr>
<td>MRS</td>
<td>methicillin-resistant staphylococci</td>
</tr>
<tr>
<td>MRSA</td>
<td>methicillin-resistant \textit{Staphylococcus aureus}</td>
</tr>
<tr>
<td>MSDS</td>
<td>material safety data sheets</td>
</tr>
<tr>
<td>NAD</td>
<td>nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>P-80</td>
<td>polysorbate 80</td>
</tr>
<tr>
<td>PBP2a</td>
<td>penicillin-binding protein 2a</td>
</tr>
<tr>
<td>PK-PD</td>
<td>pharmacokinetic-pharmacodynamic</td>
</tr>
<tr>
<td>QA</td>
<td>quality assurance</td>
</tr>
<tr>
<td>QC</td>
<td>quality control</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>US</td>
<td>United States</td>
</tr>
<tr>
<td>VISA</td>
<td>vancomycin-intermediate \textit{Staphylococcus aureus}</td>
</tr>
<tr>
<td>VRE</td>
<td>vancomycin-resistant enterococci</td>
</tr>
</tbody>
</table>
5 Indications for Performing Susceptibility Tests

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. Mechanisms of resistance include production of drug-inactivating enzymes, alteration of drug targets, and altered drug uptake or efflux. Some organisms have predictable susceptibility to antimicrobial agents, and empiric therapy for these organisms is widely accepted. Susceptibility tests are seldom necessary when the infection is due to a microorganism recognized as susceptible to a highly effective drug (eg, the continued susceptibility of *Streptococcus pyogenes* to penicillin). For *S. pyogenes* isolates from penicillin-allergic patients, erythromycin or another macrolide may be tested to detect strains resistant to those agents. Susceptibility tests are also important in studies of the epidemiology of resistance and in studies of new antimicrobial agents.

Isolated colonies of each type of organism that may be pathogenic should be selected from primary agar plates and tested individually for susceptibility. Identification procedures are often performed at the same time. Mixtures of different types of microorganisms should not be tested on the same susceptibility test plate or panel. The practice of conducting susceptibility tests directly with clinical material (eg, normally sterile body fluids and urine) should be avoided except in clinical emergencies when the direct Gram stain suggests a single pathogen. When testing has been carried out directly with the clinical material, results should be reported as preliminary, and the susceptibility test must be repeated using the standardized methodology.

When the nature of the infection is not clear and the specimen contains mixed growth or normal flora, in which the organisms probably bear little relationship to the infectious process treated, susceptibility tests are often unnecessary, and the results may be misleading.

The MIC obtained using a dilution test may tell a physician the concentration of antimicrobial agent required at the site of infection to inhibit the infecting organism. The MIC, however, does not represent an absolute value. The “true” MIC is somewhere between the lowest test concentration that inhibits the organism’s growth (that is, the MIC reading) and the next lower test concentration. If, for example, twofold dilutions were used and the MIC is 16 μg/mL, the “true” MIC would be between 16 μg/mL and 8 μg/mL. Even under the best of controlled conditions, a dilution test may not yield the same end point each time it is performed. Generally, the acceptable reproducibility of the test is within one twofold dilution of the actual end point. To avoid greater variability, the dilution test must be standardized and carefully controlled as described herein.

MICs have been determined using concentrations derived traditionally from serial twofold dilutions indexed to the base 2 (eg, 1, 2, 4, 8, 16 μg/mL). Other dilution schemes have also been used, including use of as few as two widely separated or “breakpoint” concentrations or concentrations between the usual values (eg, 4, 8, 12, 16 μg/mL). The results from these alternative methods may be equally useful clinically; however, some are more difficult to control (see Section 16.3). When there is inhibition of growth at the lowest concentration tested, the true MIC value cannot be accurately determined and should be reported as equal to or less than the lowest concentration tested. To apply interpretive criteria when concentrations between the usual dilutions are tested, results falling between serial twofold dilution of the actual end point. To avoid greater variability, the dilution test must be standardized and carefully controlled as described herein.

Whenever MIC results are reported to clinicians to direct therapy, an interpretive category (ie, susceptible, intermediate, or resistant) should accompany the MIC result based on the criteria outlined in M100 Tables 2A through 2L. When tests in which four or fewer consecutive concentrations are tested or when nonconsecutive concentrations are tested, an interpretive category result must be reported. The MIC result may also be reported if desired.

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6 Selection of Antimicrobial Agents for Routine Testing and Reporting

Selection of the most appropriate antimicrobial agents to test and to report is a decision best made by each clinical laboratory in consultation with the infectious disease practitioners and the pharmacy, as well as the pharmacy and therapeutics and infection control committees of the medical staff. The recommendations in M100 Tables 1 and 1A for each organism group list agents of proven efficacy that show acceptable in vitro test performance. Considerations in the assignment of agents to specific test/report groups include clinical efficacy, prevalence of resistance, minimizing emergence of resistance, cost, FDA clinical indications for usage, and current consensus recommendations for first choice and alternative drugs. Tests of selected agents may be useful for infection control purposes.

6.1 Routine Reports

The lists of agents in M100 Tables 1 and 1A are those considered appropriate at present for routine testing and for reporting. To avoid misinterpretation, routine reports to physicians should include only those antimicrobial agents appropriate for therapeutic use, as suggested in M100 Tables 1 and 1A. Agents may be added to or removed from these basic lists as conditions demand. Antimicrobial agents other than those appropriate for use in therapy may also be tested to provide taxonomic data and epidemiologic information, but they should not be included on patient reports. However, such results should be available in the laboratory for the use of the infection control practitioner and/or hospital epidemiologist.

6.2 Nonproprietary Names

To minimize confusion, all antimicrobial agents should be reported using official nonproprietary (ie, generic) names. To emphasize the relatedness of the many currently available antimicrobial agents, they may be grouped together by drug classes as follows:

6.2.1 β-Lactams (see M100 Glossary I, Part 1)

β-lactam antimicrobial agents all share the common, central, four-member β-lactam ring inhibition of cell wall synthesis as the primary mode of action. Additional ring structures or substituent groups added to the β-lactam ring determine whether the agent is classified as a penicillin, cephem, carbapenem, or monobactam.

6.2.1.1 Penicillins

Penicillins are primarily active against non-β-lactamase-producing, aerobic gram-positive, some fastidious, aerobic gram-negative bacteria, and some anaerobic bacteria. Aminopenicillins (ampicillin and amoxicillin) are active against additional gram-negative species, including some members of the Enterobacteriaceae. Carboxypenicillins (carbenicillin and ticarcillin) and ureidopenicillins (mezlocillin and piperacillin) are active against an expanded list of gram-negative bacteria, including many Pseudomonas and Burkholderia spp. Penicillinase-stable penicillins (cloxacillin, dicloxacillin, methicillin, nafcillin, and oxacillin) are active against predominantly gram-positive bacteria, including penicillinase-producing staphylococci.

6.2.1.2 β-Lactam/β-Lactamase Inhibitor Combinations

These antimicrobial agents are combinations that include a penicillin and a second agent that has minimal antibacterial activity, but functions as an inhibitor of some β-lactamases. Currently, three β-lactamase inhibitors are in use: clavulanic acid, sulbactam, and tazobactam. The results of tests of only the penicillin
portion of the combination against β-lactamase-producing organisms are often not predictive of susceptibility to the two-drug combination.

6.2.1.3 Cephems (including cephalosporins)

Different cephem antimicrobial agents exhibit somewhat different spectrums of activity against aerobic and anaerobic gram-positive and gram-negative bacteria. The cephem antimicrobial class includes the classical cephapensprins, as well as the agents in subclasses cephymcin, oxacephem, and carbacephems (see M1007 Glossary I). Cephalosporins are often referred to as “first-,” “second-,” “third-,” or “fourth-generation” cephapensprins, based on the extent of their activity against the more antimicrobial agent-resistant, gram-negative aerobic bacteria. Not all representatives of a specific group or generation necessarily have the same spectrum of activity. Because of these differences in activities, representatives of each group may be selected for routine testing.

6.2.1.4 Penems

The penem antimicrobial class, which includes two subclasses, the carbapenems and penems, differs slightly in structure from penicillins; agents in this class are much more resistant to β-lactamase hydrolysis, which provides them with broad-spectrum activity against many gram-positive and gram-negative bacteria.

6.2.1.5 Monobactams

Monobactam antimicrobial agents are monocyclic β-lactams. At present, aztreonam, which has activity only against gram-negative aerobic bacteria, is the only monobactam antimicrobial agent approved for use by the FDA.

6.2.2 Non-β-lactams (see M1007 Glossary I, Part 2)

6.2.2.1 Aminoglycosides

Aminoglycosides are structurally related antimicrobial agents that inhibit bacterial protein synthesis at the ribosomal level. This class includes agents variously affected by aminoglycoside-inactivating enzymes, resulting in some differences in the spectrum of activity among the agents. Aminoglycosides are used primarily to treat aerobic gram-negative rod infections or in synergistic combinations with cell-wall-active antimicrobial agents (eg, penicillin, ampicillin, and vancomycin) against some resistant, gram-positive bacteria, such as enterococci.

6.2.2.2 Folate Pathway Inhibitors

Sulfonamides and trimethoprim are chemotherapeutic agents with similar spectra of activity resulting from the inhibition of the bacterial folate pathway. Sulfisoxazole is among the most commonly used sulfonamides in the treatment of urinary tract infections; thus, it may be the appropriate selection for in vitro testing. Sulfamethoxazole is usually tested in combination with trimethoprim, because these two antimicrobial agents inhibit sequential steps in the folate pathway of some gram-positive and gram-negative bacteria.

6.2.2.3 Glycopeptides

Glycopeptide antimicrobial agents, which include vancomycin (in the glycopeptide subclass) and teicoplanin (in the lipoglycopeptide subclass), share a complex chemical structure and a principal mode of action of inhibition of cell wall synthesis at a different site than that of the β-lactams. The activity of this group is directed primarily at aerobic gram-positive bacteria. Vancomycin is an accepted agent for
6.2.2.4 Lipopeptides

Lipopeptides are a structurally related group of antimicrobial agents whose principal target is the cell membrane. The polymyxin subclass, which includes polymyxin B and colistin, has activity against gram-negative organisms. Daptomycin is a cyclic lipopeptide with activity against gram-positive organisms. Lipopeptide activity is strongly influenced by the presence of divalent cations in the medium used to test them. The presence of excess calcium cations inhibits the activity of the polymyxins, whereas the presence of physiologic levels (50 mg/L) of calcium ions is essential for the proper activity of daptomycin.

6.2.2.5 Macrolides

Macrolides are structurally related antimicrobial agents that inhibit bacterial protein synthesis at the ribosomal level. Several members of this class currently in use may need to be considered for testing against fastidious, gram-negative bacterial isolates. For gram-positive organisms, only erythromycin may need to be tested routinely. The macrolide group of antimicrobials consists of several subgroups that include azithromycin, clarithromycin, dirithromycin, and the ketolide telithromycin.

6.2.2.6 Quinolones

Quinolones (quinolones and fluoroquinolones) are structurally related antimicrobial agents that function primarily by inhibiting the DNA-gyrase or topoisomerase activity of many gram-positive and gram-negative bacteria. Some differences in spectrum may require separate testing of the individual agents.

6.2.2.7 Tetracyclines

Tetracyclines are structurally related antimicrobial agents that inhibit protein synthesis at the ribosomal level of certain gram-positive and gram-negative bacteria. Agents in this group are closely related and, with few exceptions, only tetracycline may need to be tested routinely. Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. However, some organisms that are intermediate or resistant to tetracycline may be susceptible to doxycycline or minocycline or both. Tigecycline, a glycylcycline, is a derivative of minocycline that has activity against organisms that may be resistant to other tetracyclines.

6.2.2.8 Single-Drug Classes

The following antimicrobial agents (antimicrobial class) are currently the only members of their respective classes used in humans that are included in this document and are appropriate for in vitro testing. These include chloramphenicol (phenicols), clindamycin (lincosamides), linezolid (oxazolidinones), quinupristin-dalfopristin (streptogramins), telithromycin (ketolides), and tigecycline (glycylcyclines), all of which inhibit protein synthesis, and rifampin (ansamycins), which is a ribonucleic acid (RNA) synthesis inhibitor. Nitrofurantoin (nitrofurans), which is used only in the therapy of urinary tract infections, acts by inhibiting several protein synthesis and assembly steps at the ribosomal level. Fosfomycin (fosfomycins), also approved by the FDA for urinary tract infections, inhibits enzymes involved in cell wall synthesis.
6.3 Selection Guidelines

To make routine susceptibility testing relevant and practical, the number of agents tested should be limited. M1007 Tables 1 and 1A list those agents that fulfill the basic requirements for routine use in most clinical laboratories. The tables are divided into columns based on specific organisms or organism groups, and then the various drugs are indicated in priority for testing to assist laboratories in the selection of their routine testing batteries.

The listing of drugs together in a single box designates clusters of agents for which interpretive results (susceptible, intermediate, or resistant) and clinical efficacy are similar. Within each box, an “or” between agents designates those agents for which cross-resistance and cross-susceptibility are nearly complete. This means combined major and very major errors are fewer than 3% and minor errors are fewer than 10%, based on a large population of bacteria tested. In addition, to qualify for an “or,” at least 100 strains with resistance to the agents in question must be tested and a result of “resistant” must be obtained with all agents for at least 95% of the strains. “Or” is also used for comparable antimicrobial agents when tested against organisms for which “susceptible-only” interpretive criteria are provided (eg, cefotaxime or ceftriaxone with Haemophilus influenzae). Thus, results from one agent connected by an “or” could be used to predict results for the other agent. For example, a non-ESBL-producing isolate of Enterobacteriaceae susceptible to cefotaxime can be considered susceptible to ceftriaxone. The results obtained from testing cefotaxime would be reported and a comment could be included on the report that the isolate is also susceptible to ceftriaxone. When no “or” connects agents within a box, testing of one agent cannot be used to predict results for another either owing to discrepancies or insufficient data.

6.4 Suggested Guidelines for Routine and Selective Testing and Reporting

As listed in M1007 Tables 1 and 1A, agents in Group A are considered appropriate for inclusion in a routine, primary testing panel and for routine reporting of results for the specified organism groups.

Group B comprises agents that may warrant primary testing. However, they may be reported only selectively, such as when the organism is resistant to agents of the same class, as in Group A. Other indications for reporting the result might include a selected specimen source (eg, a third-generation cephalosporin for enteric bacilli from cerebrospinal fluid [CSF] or trimethoprim-sulfamethoxazole for urinary tract isolates); a polymicrobial infection; infections involving multiple sites; cases of patient allergy, intolerance, or failure to respond to an agent in Group A; or for purposes of infection control.

Group C comprises alternative or supplemental antimicrobial agents that may require testing in those institutions that harbor endemic or epidemic strains resistant to several of the primary drugs (especially in the same class, eg, β-lactams or aminoglycosides); for treatment of patients allergic to primary drugs; for treatment of unusual organisms (eg, chloramphenicol for extraintestinal isolates of Salmonella spp.); or for reporting to infection control as an epidemiologic aid.

Group U lists certain antimicrobial agents (eg, nitrofurantoin and certain quinolones) that are used only or primarily for treating urinary tract infections; these agents should not be routinely reported against pathogens recovered from other sites of infection. Other agents with broader indications may be included in Group U for specific urinary pathogens (eg, Pseudomonas aeruginosa).

Group O (“other”) includes agents that have a clinical indication for the organism group, but are generally not candidates for routine testing and reporting in the United States.

Group Inv. (“investigational”) agents are undergoing clinical investigation for the organism group and have not yet been approved by the FDA for use in the United States.
Each laboratory should decide which agents in M100 Tables 1 and 1A to report routinely (Group A) and those that might be reported only selectively (Group B) in consultation with the infectious disease practitioners, the pharmacy, as well as the pharmacy and therapeutics and infection control committees of the health care institution. Selective reporting should help improve the clinical relevance of test reports and help minimize the selection of multiresistant, health care-associated strains by overuse of broad-spectrum agents. Results for Group B agents not reported routinely should be available on request, or they may be reported for selected specimens. When confirmed, unexpected resistance should be reported (eg, resistance to a secondary agent but susceptibility to a primary agent).

7  Antimicrobial Agents

7.1  Source

Obtain antimicrobial standards or reference powders directly from the drug manufacturer, from the United States Pharmacopoeia (www.usp.org), or from certain other commercial sources. Parenteral preparations should not be used for susceptibility testing. Acceptable powders bear a label that states the drug’s generic name, lot number, potency (usually expressed in micrograms [μg] or International Units [IU] per milligram of powder), and expiration date. Store the powders as recommended by the manufacturer or at \( \leq -20 ^\circ C \) in a desiccator (preferably in a vacuum). When the desiccator is removed from the refrigerator or freezer, allow the desiccator to warm to room temperature before opening to avoid condensation of water.

7.2  Weighing Antimicrobial Powders

All antimicrobial agents are assayed for standard units of activity. The assay units may differ widely from the actual weight of the powder and often may differ between drug production lots. Thus, a laboratory must standardize its antimicrobial solutions based on assays of the lots of antimicrobial powders that are used to make stock solutions.

The value for potency supplied by the manufacturer should include consideration of measures of purity (usually by high-performance liquid chromatography [HPLC] assay), water content (eg, by Karl Fischer analysis or by weight loss on drying), and the salt/counter-ion fraction (if the compound is supplied as a salt instead of free acid or base). The potency may be expressed as a percentage, or in units of μg/mg (w/w).

In some cases, a certificate of analysis with values for each of these components may be provided with antimicrobial agent powders; in this case, an overall value for potency may not be provided, but can be calculated from HPLC purity, water content, and, when applicable, the active fraction for drugs supplied as a salt (eg, hydrochloride, mesylate). However, when applying these calculations, if any value is unknown or is not clearly determined from the certificate of analysis, it is advisable that the factors used in this calculation be confirmed with the supplier or the manufacturer. The following demonstrates an example calculation:

Example: meropenem trihydrate

Certificate of analysis data:
Assay purity (by HPLC): 99.8%
Measured water content (by Karl Fischer analysis): 12.1% (w/w)
Active fraction: 100% (supplied as the free acid and not a salt)

Potency calculation from above data:
Potency = (Assay purity) \( \times (Active \ fraction) \times (1 - Water \ Content) \)
Potency = \( (998) \times (1.0) \times (1 - 0.121) \)
Potency = 877 μg/mg or 87.7%
Use either of the following formulas to determine the amount of powder (1) or diluent (2) needed for a standard solution:

\[
Weight \ (\text{mg}) = \frac{Volume \ (\text{mL}) \cdot Concentration \ (\mu\text{g/mL})}{Potency \ (\mu\text{g/mg})}
\]  

or

\[
Volume \ (\text{mL}) = \frac{Weight \ (\text{mg}) \cdot Potency \ (\mu\text{g/mg})}{Concentration \ (\mu\text{g/mL})}
\]

Weigh the antimicrobial powder on an analytical balance that has been calibrated by approved reference weights from a national metrology organization. If possible, weigh more than 100 mg of powder. It is advisable to accurately weigh a portion of the antimicrobial agent in excess of that required and to calculate the volume of diluent needed to obtain the final concentration desired as in formula 2 above.

Example: To prepare 100 mL of a stock solution containing 1280 μg/mL of antimicrobial agent with antimicrobial powder that has a potency of 750 μg/mg, 170 mg to 200 mg of the antimicrobial powder should be accurately weighed. If the actual weight is 182.6 mg, the volume of diluent needed is as follows:

\[
Volume \ (\text{mL}) = \frac{(Actual \ Weight) \cdot (Potency)}{(Desired \ Concentration)} = \frac{182.6 \text{ mg} \cdot 750 \mu\text{g/mg}}{1280 \mu\text{g/mL}} = 107.0 \text{ mL}
\]

Therefore, dissolve 182.6 mg of antimicrobial powder in 107.0 mL of diluent.

7.3 Preparing Stock Solutions

Prepare antimicrobial agent stock solutions at concentrations of at least 1000 μg/mL (example: 1280 μg/mL) or 10 times the highest concentration to be tested, whichever is greater. Some antimicrobial agents, however, have limited solubility and may require preparation of lower stock concentrations. In all cases, consider directions provided by the drug’s manufacturer as part of determining solubility.

Some drugs must be dissolved in solvents other than water. In such cases:

- Use a minimum amount of solvent to solubilize the antimicrobial powder.
- Finish diluting the final stock concentration with water or the appropriate diluent as indicated in M1007 Table 5.
- For potentially toxic solvents, consult the material safety data sheets (MSDS) available from the manufacturer (see M1007 Table 5).

Because microbial contamination is extremely rare, solutions that have been prepared aseptically but not filter sterilized are generally acceptable. If desired, however, solutions may be sterilized by membrane filtration. Paper, asbestos, or sintered glass filters, which may adsorb appreciable amounts of certain antimicrobial agents, must not be used. Whenever filtration is used, it is important to document the absence of adsorption by appropriate assay procedures.
Dispense small volumes of the sterile stock solutions into sterile glass, polypropylene, polystyrene, or polyethylene vials; carefully seal; and store (preferably at −60 °C or below, but never at a temperature warmer than −20 °C and never in a self-defrosting freezer). Vials may be thawed as needed and used the same day. Any unused stock solution should be discarded at the end of the day. Stock solutions of most antimicrobial agents can be stored at −60 °C or below for six months or more without significant loss of activity. In all cases, directions provided by the drug’s manufacturer must be considered in addition to these general recommendations. Any significant deterioration of an antimicrobial agent should be reflected in the results of susceptibility testing using QC strains.

7.4 Number of Concentrations Tested

The concentrations tested for a particular antimicrobial agent should encompass the interpretive breakpoints shown in M100® Tables 2A through 2L, but the actual number of concentrations tested is the decision of the laboratory. However, it is advisable to choose a range that allows at least one QC organism to have on-scale values. Unusual concentrations may be tested for special purposes (eg, high concentrations of gentamicin and streptomycin may be tested to indicate a synergistic effect with a penicillin or glycopeptide against enterococci).

8 Inoculum Preparation for Dilution Tests

8.1 Turbidity Standard for Inoculum Preparation

To standardize the inoculum density for a susceptibility test, use a BaSO₄ turbidity standard equivalent to a 0.5 McFarland standard or its optical equivalent (eg, latex particle suspension). Prepare a BaSO₄ 0.5 McFarland standard as described in Appendix B. Alternatively, a photometric device can be used.

8.2 Direct Colony Suspension Method

The direct colony suspension method is the most convenient method for inoculum preparation. This method can be used with most organisms and is the recommended method for testing the fastidious organisms, Haemophilus spp., Neisseria gonorrhoeae, N. meningitidis, and streptococci (see Section 11), and for testing staphylococci for potential methicillin or oxacillin resistance.

(1) Prepare the inoculum by making a direct broth or saline suspension of isolated colonies selected from an 18- to 24-hour agar plate (use a nonselective medium, such as blood agar).

(2) Adjust the suspension to achieve a turbidity equivalent to a 0.5 McFarland turbidity standard. This results in a suspension containing approximately 1 to 2 x 10⁸ colony forming units (CFU)/mL for Escherichia coli ATCC®a 25922. To perform this step accurately, use either a photometric device or, if performed visually, use adequate light to visually compare the inoculum tube and the 0.5 McFarland standard against a card with a white background and contrasting black lines.

8.3 Growth Method

The growth method can be used alternatively and is sometimes preferable when colony growth is difficult to suspend directly and a smooth suspension cannot be made. It can also be used for nonfastidious organisms (except staphylococci) when fresh (24-hour) colonies, as required for the direct colony suspension method, are not available.

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a ATCC is a registered trademark of the American Type Culture Collection.
(1) Select at least three to five well-isolated colonies of the same morphologic type from an agar plate culture. Touch the top of each colony with a loop or sterile swab and transfer the growth into a tube containing 4 mL to 5 mL of a suitable broth medium, such as tryptic soy broth.

(2) Incubate the broth culture at 35 ± 2 °C until it achieves or exceeds the turbidity of the 0.5 McFarland standard (usually two to six hours).

(3) Adjust the turbidity of the actively growing broth culture with sterile saline or broth to achieve a turbidity equivalent to that of a 0.5 McFarland standard. This results in a suspension containing approximately 1 to 2 x 10^8 CFU/mL for E. coli ATCC® 25922. To perform this step accurately, use either a photometric device or, if performed visually, use adequate light to visually compare the inoculum tube and the 0.5 McFarland standard against a card with a white background and contrasting black lines.

9 Agar Dilution Procedure

The agar dilution method for determining antimicrobial susceptibility is a well-established technique. The antimicrobial agent is incorporated into the agar medium, with each plate containing a different concentration of the agent. The inocula can be applied rapidly and simultaneously to the agar surfaces using an inoculum-replicating apparatus capable of transferring 32 to 36 inocula to each plate.

The general procedure for performing agar dilution is given in this section. Variations for testing fastidious organisms are given in Section 11.

9.1 Reagents and Materials

9.1.1 Mueller-Hinton Agar

Of the many media available, the subcommittee considers MHA the best for routine susceptibility testing of nonfastidious bacteria for the following reasons:

- It shows acceptable batch-to-batch reproducibility for susceptibility testing.
- It is low in inhibitors that affect sulfonamide, trimethoprim, and tetracycline susceptibility test results.
- It supports satisfactory growth of most pathogens.
- Large bodies of data and experience have been collected about susceptibility tests performed with this medium.

Although MHA is generally reliable for susceptibility testing, results obtained with some batches may, on occasion, vary significantly. Prepare MHA as described in Appendix B. Only MHA formulations that have been tested according to and that meet the acceptance limits described in CLSI document M06 should be used.

(1) Performance test new lots of medium before use as outlined in Section 16.5.

(2) See Appendix B for instructions for preparation of MHA.

(3) Check the pH of each batch of MHA as described in Appendix B.

(4) Additions to MHA that must be made for testing specific antimicrobial agents are:
• 2% NaCl when testing staphylococci and oxacillin, nafcillin, and methicillin; and
• 25 µg/mL of glucose-6-phosphate when testing fosfomycin.

(5) Do not add calcium or magnesium cations to MHA.

9.1.2 Agar Media for Testing Fastidious Organisms

Media for testing fastidious organisms using methods described in this document are:

• MHA with 5% sheep blood;
• MHA with 5% aged sheep blood (≥ 2 weeks old) (for testing Helicobacter pylori);
• Haemophilus Test Medium (HTM); and
• GC agar base + 1% defined growth supplement.

See Appendix B for instructions for preparation of these media.

9.1.3 Inoculum Replicators

Most inoculum replicators currently available transfer 32 to 36 inocula to each plate. Replicators with pins 3 mm in diameter deliver approximately 2 µL (range 1 µL to 3 µL) onto the agar surface. Those with smaller 1-mm pins deliver tenfold less, approximately 0.1 µL to 0.2 µL.

9.2 Preparing Agar Dilution Plates

9.2.1 Dilution Scheme for Reference Method

Prepare intermediate (10x) antimicrobial agent solutions by making successive 1:2, 1:4, and 1:8 dilutions using the dilution format described in M100 Table 6 or by making serial twofold dilutions. Then, add one part of the 10x antimicrobial solution to nine parts of molten agar.

9.2.2 Procedure

(1) Add appropriate dilutions (see Section 9.2.1) of antimicrobial solution to molten test agars that have been allowed to equilibrate in a water bath to 45 °C to 50 °C.

(2) Mix the agar and antimicrobial solution thoroughly and pour into petri dishes on a level surface to result in an agar depth of 3 mm to 4 mm.

(3) Pour the plates quickly after mixing to prevent cooling and partial solidification in the mixing container. Avoid generating bubbles when mixing.

(4) Allow the agar to solidify at room temperature, and use the plates either immediately or store them in sealed plastic bags at 2 °C to 8 °C for up to five days for reference work, or longer for routine tests. In one study, agar plates containing cefaclor had to be prepared within 48 hours of use because of degradation of the drug, whereas cefamandole remained stable for greater than the recommended five days. In addition to cefaclor, other antimicrobial agents that are particularly labile are ampicillin, methicillin, imipenem, and clavulanic acid.

NOTE: Do not assume all antimicrobial agents will maintain their potency under these storage conditions. The user should evaluate the stability of plates from results obtained with QC strains and should develop applicable shelf-life criteria. This information is sometimes available from pharmaceutical manufacturers.
(5) Allow plates stored at 2 °C to 8 °C to equilibrate to room temperature before use. Make certain that the agar surface is dry before inoculating the plates. If necessary, place the plates in an incubator or laminar flow hood for approximately 30 minutes with their lids ajar to hasten drying of the agar surface.

9.2.3 Quality Control Frequency

Although weekly QC, as described in this standard, is acceptable for most drug/agar combinations, some may require more frequent testing (eg, the labile drugs described in Section 9.2.2 above). See Section 16 for further details.

9.2.4 Control Plates

Use drug-free plates prepared from the base medium (with or without supplements as indicated in Section 9.1) as growth controls.

9.3 Preparing the Inoculum

9.3.1 Inoculum Preparation

Prepare a standardized inoculum for the agar dilution method by either growing microorganisms to the turbidity of the 0.5 McFarland standard or suspending colonies directly to achieve the same density as described in Section 8. Preparation of the initial inoculum and final dilution may vary for certain fastidious organisms such as *N. meningitidis* (see Section 11 and Appendix C).

9.3.2 Dilution of Inoculum Suspension

Cultures adjusted to the 0.5 McFarland standard contain approximately 1 to 2 × 10^8 CFU/mL with most species, and the final inoculum required is 10^4 CFU per spot of 5 mm to 8 mm in diameter. When using replicators with 3-mm pins that deliver 2 μL, dilute the 0.5 McFarland suspension 1:10 in sterile broth or saline to obtain a concentration of 10^7 CFU/mL. The final inoculum on the agar will be approximately 10^4 CFU per spot. When using replicators with 1-mm pins that deliver 0.1 μL to 0.2 μL, do not dilute the initial suspension. Optimally, use the adjusted suspensions for final inoculation within 15 minutes of preparation.

9.4 Inoculating Agar Dilution Plates

(1) Arrange the tubes containing the adjusted and diluted bacterial suspensions (10^7 CFU/mL) in order in a rack. Place an aliquot of each well-mixed suspension into the corresponding well in the replicator inoculum block.

(2) Mark the agar plates for orientation of the inoculum spots.

(3) Apply an aliquot of each inoculum to the agar surface, either with an inocula-replicating device or with standardized loops or pipettes. Appropriate dilution of the inoculum suspension should be made depending on the volume of inoculum delivery so as to obtain a final concentration of 10^4 CFU/spot (see Section 9.3.2).

(4) Inoculate a growth-control plate (no antimicrobial agent) first and then, starting with the lowest concentration, inoculate the plates containing the different antimicrobial concentrations. Inoculate a second growth control plate last to ensure there was no contamination or significant antimicrobial carry-over during the inoculation.
5. Streak a sample of each inoculum on a suitable nonselective agar plate and incubate overnight to detect mixed cultures and to provide freshly isolated colonies in case retesting proves necessary.

### 9.5 Incubating Agar Dilution Plates

1. Allow the inoculated plates to stand at room temperature until the moisture in the inoculum spots has been absorbed into the agar, i.e., until the spots are dry, but no more than 30 minutes. Invert the plates and incubate at 35 ± 2 °C for 16 to 20 hours (see Sections 11 and 12 and Appendix C for exceptions).

2. Do not incubate the plates in an atmosphere with increased CO₂ when testing nonfastidious organisms, because the surface pH may be altered. However, incubate *N. gonorrhoeae*, streptococci, and *N. meningitidis* in an atmosphere containing 5% CO₂. (See Section 11, Appendix C, and M100 Tables 2A through 2L.)

### 9.6 Determining Agar Dilution End Points

1. Place the plates on a dark, nonreflecting surface to determine the end points. Record the MIC as the lowest concentration of antimicrobial agent that completely inhibits growth, disregarding a single colony or a faint haze caused by the inoculum. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, read the end point at the concentration in which there is 80% or greater reduction in growth as compared to the control.

2. If two or more colonies persist in concentrations of the agent beyond an obvious end point, or if there is no growth at lower concentrations but growth at higher concentrations, check the culture purity and repeat the test if required.

### 10 Broth Dilution Procedures (Macrodilution and Microdilution)

The general procedures for performing broth dilution are given in this section. Variations for testing fastidious organisms are given in Section 11.

#### 10.1 Mueller-Hinton Broth

Mueller-Hinton broth is recommended as the medium of choice for susceptibility testing of commonly isolated, rapidly growing aerobic or facultative organisms because:

- it shows acceptable batch-to-batch reproducibility for susceptibility testing;
- it is low in inhibitors that affect sulfonamide, trimethoprim, and tetracycline susceptibility test results;
- it supports satisfactory growth of most pathogens; and
- a large body of data and experience has been gathered about tests performed with this medium.

1. The medium of choice for routine broth dilution testing is cation-adjusted MHB (CAMHB). Instructions for preparation are given in Appendix B.

2. Check the pH of each batch of MHB as described in Appendix B.

3. Evaluate the MIC performance characteristics of each batch of broth using a standard set of QC organisms (see Section 16.3). If a new lot of MHB does not yield the expected MICs, the cation content should be investigated along with other variables and components of the test.

4. To determine the suitability of the medium for sulfonamide and trimethoprim tests, perform MICs with *Enterococcus faecalis* ATCC® 29212. The end points should be easy to read (as 80% or greater
reduction in growth as compared to the control). If the MIC for trimethoprim-sulfamethoxazole is \( \leq 0.5/9.5 \) \( \mu g/mL \), the medium may be considered adequate.

(5) Additions to CAMHB that must be made for testing specific antimicrobial agents are:

- 2% NaCl when testing oxacillin and staphylococci;
- 0.002% polysorbate-80 (P-80) when testing dalbavancin and oritavancin; and
- additional calcium to equal 50 mg/L when testing daptomycin.

### 10.2 Broth Media for Fastidious Organisms

Media that can be used for testing fastidious organisms using methods described in this document are:

- CAMHB + 2.5% to 5% lysed horse blood (LHB); and
- HTM broth.

Instructions for preparation of these media are given in Appendix B.

### 10.3 Macrodilution (Tube) Broth Method

#### 10.3.1 Preparing and Storing Diluted Antimicrobial Agents

(1) Use sterile 13- x 100-mm test tubes to conduct the test. If the tubes are to be saved for later use, be sure they can be frozen.

(2) Close the tubes with loose screw-caps, plastic or metal closure caps, or cotton plugs.

(3) Use a growth control tube containing broth without antimicrobial agent for each organism tested.

(4) Prepare the final twofold (or other) dilutions of antimicrobial agent volumetrically in the broth. The schedule in M100 Tables 7 and 7A provides a convenient and reliable procedure for preparing dilutions. A minimum final volume of 1 mL of each dilution is needed for the test. Use one pipette for measuring all diluents and then for adding the stock antimicrobial solution to the first tube. For each subsequent dilution step, use a new pipette. Because there is a 1:2 dilution of the drugs when an equal volume of inoculum is added, the antimicrobial dilutions are often prepared at double the desired final concentration.

(5) Use the tubes on the day of preparation or immediately place them in a freezer at \( \leq -20 \) °C (preferably at \( \leq -60 \) °C) until needed. Although the antimicrobial agents in frozen tubes usually remain stable for several months, certain agents (eg, clavulanic acid and imipenem) are more labile than others and should be stored at \( \leq -60 \) °C. Do not store the tubes in a self-defrosting freezer. Thawed antimicrobial solutions must not be refrozen; repeated freeze-thaw cycles accelerate the degradation of some antimicrobial agents, particularly \( \beta \)-lactams.

#### 10.3.2 Inoculum Preparation and Inoculation

(1) Prepare a standardized inoculum using either the direct colony suspension or growth method as described in Section 8.

(2) Optimally within 15 minutes of preparation, dilute the adjusted inoculum suspension in broth so, after inoculation, each tube contains approximately 5 x 10^5 CFU/mL. This can be accomplished by diluting...
the 0.5 McFarland suspension 1:150, resulting in a tube containing approximately 1 x 10^6 CFU/mL. The subsequent 1:2 dilution in step 3 brings the final inoculum to 5 x 10^5 CFU/mL.

(3) Within 15 minutes after the inoculum has been standardized as described above, add 1 mL of the adjusted inoculum to each tube containing 1 mL of antimicrobial agent in the dilution series (and a positive control tube containing only broth), and mix. This results in a 1:2 dilution of each antimicrobial concentration and a 1:2 dilution of the inoculum. It is advisable to perform a purity check of the inoculum suspension by subculturing an aliquot onto a nonselective agar plate for simultaneous incubation.

10.4 Broth Microdilution Method

This method is called “microdilution,” because it involves the use of small volumes of broth dispensed in sterile, plastic microdilution trays that have round or conical bottom wells. Each well should contain 0.1 mL of broth. The broth microdilution method described in M07 is the same methodology outlined in ISO 20776-1.

10.4.1 Preparing and Storing Diluted Antimicrobial Agents

(1) To prepare microdilution trays, make intermediate twofold (or other) dilutions of antimicrobial agent volumetrically in broth or sterile water. For the intermediate (10x) antimicrobial solutions, dilute the concentrated antimicrobial stock solution (see Section 7.3) as described in M100 Table 7 or by making serial twofold dilutions. Use one pipette for measuring all diluents and then for adding the stock antimicrobial solution to the first tube. For each subsequent dilution step, use a new pipette. Dispense the antimicrobial/broth solutions into the plastic microdilution trays.

(2) The dilution scheme outlined in M100 Table 7A must be used for diluting water-insoluble agents, such as dalbavancin.

(3) The most convenient method of preparing microdilution trays is by use of a dispensing device and antimicrobial dilutions made in at least 10 mL of broth. The dispensing device then delivers 0.1 (±0.02) mL into each of the 96 wells of a standard tray.

(4) If the inoculum is added by pipette as described in Section 10.4.2 (3), prepare the antimicrobial solutions at twice the desired final concentration, and fill the wells with 0.05 mL instead of 0.1 mL. Each tray should include a growth control well and a sterility (uninoculated) well.

(5) Seal the filled trays in plastic bags and immediately place in a freezer at ≤ −20 °C (preferably at ≤ −60 °C) until needed. Although the antimicrobial agents in frozen trays usually remain stable for several months, certain agents (e.g., clavulanic acid and imipenem) are more labile than others and should be stored at ≤ −60 °C. Do not store the trays in a self-defrosting freezer. Thawed antimicrobial solutions must not be refrozen; repeated freeze-thaw cycles accelerate the degradation of some antimicrobial agents, particularly β-lactams.

10.4.2 Inoculum Preparation and Inoculation

(1) Prepare a standardized inoculum using either the direct colony suspension or growth method as described in Section 8.

(2) Optimally within 15 minutes of preparation, dilute the adjusted inoculum suspension in water, saline, or broth so, after inoculation, each well contains approximately 5 x 10^5 CFU/mL (range 2 to 8 x 10^5 CFU/mL). The dilution procedure to obtain this final inoculum varies according to the method of delivery of the inoculum to the individual wells and it must be calculated for each situation. For
microdilution tests, the exact inoculum volume delivered to the wells must be known to make this
calculation. For example, if the volume of broth in the well is 0.1 mL and the inoculum volume is
0.01 mL, then the 0.5 McFarland suspension (1 x 10⁸ CFU/mL) should be diluted 1:20 to yield 5 x
10⁶ CFU/mL. When 0.01 mL of this suspension is inoculated into the broth, the final test
concentration of bacteria is approximately 5 x 10⁵ CFU/mL (or 5 x 10⁴ CFU/well in the microdilution
method).

(3) Within 15 minutes after the inoculum has been standardized as described above, inoculate each well
of a microdilution tray using an inoculator device that delivers a volume that does not exceed 10% of
the volume in the well (e.g., ≤ 10 μL of inoculum in 0.1 mL antimicrobial agent solution). Conversely,
if a 0.05-mL pipette is used, it results in a 1:2 dilution of the contents of each well (containing 0.05 mL),
as with the macrodilution procedure.

(4) It is advisable to perform a purity check of the inoculum suspension by subculturing an aliquot onto a
nonselective agar plate for simultaneous incubation.

10.5 Colony Counts of Inoculum Suspensions

Laboratories are encouraged to perform colony counts on inoculum suspensions at least quarterly to
ensure that the final inoculum concentration routinely obtained closely approximates 5 x 10⁵ CFU/mL for
E. coli ATCC® 25922. Do this by removing a 0.01-mL aliquot from the growth control well or tube
immediately after inoculation and diluting it in 10 mL of saline (1:1000 dilution). After mixing, remove a
0.1-mL aliquot and spread it over the surface of a suitable agar medium. After incubation, the presence of
approximately 50 colonies indicates an inoculum density of 5 x 10⁵ CFU/mL.

10.6 Incubation

(1) Incubate the inoculated macrodilution tubes or microdilution trays at 35 ± 2 °C for 16 to 20 hours in
an ambient air incubator (for exceptions, see Sections 11 and 12 and Appendix C). To maintain the
same incubation temperature for all cultures, do not stack microdilution trays more than four high.

(2) To prevent drying, seal each tray in a plastic bag, with plastic tape, or with a tight-fitting plastic cover
before incubating.

(3) Incubation times may differ for fastidious organisms or some problem organisms with difficult-to-
detect resistance; follow specific instructions in Appendix C, in Sections 11 to 13, or the appropriate
table in M100.⁷

10.7 Determining MIC End Points

The MIC is the lowest concentration of antimicrobial agent that completely inhibits growth of the
organism in the tubes or microdilution wells as detected by the unaided eye. Viewing devices intended to
facilitate reading microdilution tests and recording of results may be used as long as there is no
compromise in the ability to discern growth in the wells.

(1) Compare the amount of growth in the wells or tubes containing the antimicrobial agent with the
amount of growth in the growth-control wells or tubes (no antimicrobial agent) used in each set of
tests when determining the growth end points. For a test to be considered valid, acceptable growth
(≥ 2 mm button or definite turbidity) must occur in the growth-control well.

(2) With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth;
therefore, read the end point at the concentration in which there is ≥ 80% reduction in growth as
compared to the control.
(3) When a single skipped well occurs in a microdilution test, read the highest MIC. Do not report results for drugs for which there is more than one skipped well.

(4) Generally, microdilution MICs for gram-negative bacilli are the same or one twofold dilution lower than the comparable macrodilution MICs.¹⁸

11 Fastidious Organisms

Mueller-Hinton medium described above for the rapidly growing aerobic pathogens is not adequate for susceptibility testing of fastidious organisms. If MIC tests are performed with fastidious organisms, the medium, QC procedures, and interpretive criteria must be modified to fit each organism. Dilution tests for the following organisms are described here:

- *H. influenzae* and *H. parainfluenzae*, using HTM (broth dilution only);
- *N. gonorrhoeae*, using GC agar base medium;
- Streptococci, using LHB-supplemented CAMHB; and
- *N. meningitidis*, using LHB-supplemented CAMHB or MHA with sheep blood.

Details for the conditions required for these tests are summarized in Appendix C and further explained below.

Certain fastidious bacteria other than those listed above may be tested by a dilution or disk diffusion method as described in CLSI document M45.⁵ Methods for testing anaerobic bacteria are given in CLSI document M11.⁴

11.1 *Haemophilus influenzae* and *H. parainfluenzae*

When the designation *Haemophilus* spp. is used below, it applies only to these two species. MIC testing of *H. influenzae* and *H. parainfluenzae* using HTM¹⁹ has been developed only for broth dilution as described below. The agar dilution method using HTM has not been validated. Details of the medium preparation are described in Appendix B. If sulfonamides or trimethoprim are tested, add 0.2 IU thymidine phosphorylase to the medium aseptically. The pH should be 7.2 to 7.4.

*H. influenzae* ATCC® 10211 is recommended as a useful additional QC strain to verify the growth promotion properties of HTM. In particular, manufacturers of HTM are encouraged to use *H. influenzae* ATCC® 10211 as a QC test strain.

11.1.1 Test Procedure

Follow the general test procedure in Section 10 with the following exceptions:

(1) Use the direct colony suspension procedure when testing *Haemophilus* spp. Using colonies taken directly from an overnight (preferably 20- to 24-hour) chocolate agar culture plate, prepare a suspension of the test organism in MHB or saline. Adjust the suspension with broth or saline using a photometric device to achieve a turbidity equivalent to a 0.5 McFarland standard. Suspensions prepared from a 20- to 24-hour agar plate contain approximately 1 to 2 \( \times 10^8 \) CFU/mL, while those prepared from a 16- to 18-hour plate contain more cells (approximately 3 to 4 \( \times 10^8 \) CFU/mL). Exercise care in preparing this suspension, taking into account the age of the plate from which the inoculum is prepared; inoculum concentrations higher than 5 \( \times 10^8 \) CFU/mL may lead to false-resistant results with some β-lactam antimicrobial agents, particularly when β-lactamase-producing strains of *H. influenzae* are tested. Use the suspension for tray inoculation within 15 minutes after adjusting the turbidity.

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(2) Incubate the trays or tubes at 35 ± 2 °C in ambient air for 20 to 24 hours before reading the MICs.

11.1.2 MIC Interpretive Criteria

The antimicrobial agents suggested for routine testing of Haemophilus spp. are listed in M100 Table 1A. Specific MIC interpretive criteria to be used when testing Haemophilus spp. are listed in M100 Table 2E.

11.2 Neisseria gonorrhoeae

MIC testing of N. gonorrhoeae has been developed only for agar dilution using GC agar base and 1% defined growth supplement as described in Appendix B and M100 Table 2F.

11.2.1 Test Procedure

Follow the general test procedure in Section 9 with the following exceptions:

(1) Use the direct colony suspension procedure when testing N. gonorrhoeae. Using colonies taken directly from an overnight chocolate agar culture plate, prepare a suspension equivalent to that of the 0.5 McFarland standard in MHB or 0.9% phosphate buffered saline, pH 7.0. Use the suspension for plate inoculation within 15 minutes after adjusting the turbidity.

(2) Incubate the plates at 36 ± 1 °C (do not exceed 37 °C) in an atmosphere of 5% CO₂ for 20 to 24 hours before reading the MICs.

11.2.2 MIC Interpretive Criteria

The antimicrobial agents suggested for routine testing of N. gonorrhoeae are indicated in M100 Table 1A. Specific MIC interpretive criteria to be used when testing N. gonorrhoeae are listed in M100 Table 2F.

11.3 Neisseria meningitidis

Caution: Perform all antimicrobial susceptibility testing of N. meningitidis in a biological safety cabinet (BSC). Manipulating suspensions of N. meningitidis outside a BSC is associated with a high risk for contracting meningococcal disease. Laboratory-acquired meningococcal disease is associated with a case fatality rate of 50%. Exposure to droplets or aerosols of N. meningitidis is the most likely risk for laboratory-acquired infection. Rigorous protection from droplets or aerosols is mandated when microbiological procedures (including antimicrobial susceptibility testing) are performed on all N. meningitidis isolates, especially those from sterile sites (blood or CSF).

Recommended precautions: Specimens for N. meningitidis analysis and cultures of N. meningitidis not associated with invasive disease may be handled in Biosafety Level 2 (BSL-2) facilities with rigorous application of BSL-2 standard practices, special practices, and safety equipment. All sterile-site isolates of N. meningitidis should be manipulated within a BSC. If a BSC is unavailable, manipulation of these isolates should be minimized, limited to Gram staining or serogroup identification using phenolized saline solution, while wearing a laboratory coat and gloves and working behind a full face splash shield. Use Biosafety Level 3 (BSL-3) practices, procedures, and containment equipment for activities with a high potential for droplet or aerosol production and for activities involving production quantities or high concentrations of infectious materials. If BSL-2 or BSL-3 facilities are not available, forward isolates to a reference or public health laboratory with a minimum of BSL-2 facilities.

Laboratorians who are exposed routinely to potential aerosols of N. meningitidis should consider vaccination according to the current recommendations of the CDC Advisory Committee on Immunization Practices (www.cdc.gov). Vaccination decreases but does not eliminate the risk of infection, because it is
less than 100% effective and does not provide protection against serogroup B, a frequent cause of laboratory-acquired cases.

Broth microdilution and agar dilution susceptibility testing of *N. meningitidis* have been validated to provide methods for detection of possible emerging resistance. To date, resistance has mostly been found to older agents used for therapy (penicillin or ampicillin), or agents used for prophylaxis of case contacts. Because resistance to antimicrobial agents such as ceftriaxone or cefotaxime that are often employed for therapy of invasive diseases has not been detected, routine testing of isolates by clinical laboratories is not necessary.

Broth microdilution testing of *N. meningitidis* isolates is performed using CAMHB with 2% to 5% LHB; alternatively, agar dilution can be performed using MHA supplemented with 5% sheep blood. Details for preparation are given in Appendix B.

### 11.3.1 Test Procedure

Follow the general test procedures as described in Sections 9 or 10 with the following exceptions:

1. Use the direct colony suspension procedure as described in Section 8.2 when testing *N. meningitidis*. Using colonies taken directly from an overnight (20- to 24-hour) enriched chocolate agar plate incubated at 35 ± 2 °C in 5% CO₂, prepare a suspension in saline to achieve a turbidity equivalent to a 0.5 McFarland standard. Use the suspension for tray or plate inoculation within 15 minutes after adjusting the turbidity.

2. Incubate trays or agar plates at 35 ± 2 °C in 5% CO₂ for 20 to 24 hours before reading the MICs.

### 11.3.2 MIC Interpretive Criteria

Specific MIC interpretive criteria and QC organisms used when testing *N. meningitidis* are listed in Table 2J.

### 11.4 *Streptococcus pneumoniae* and Other *Streptococcus* spp.

MIC testing of *Streptococcus* spp. using CAMHB with 2.5% to 5% LHB has been developed only for broth dilution. Details of the medium preparation are described in Appendix B. The agar dilution method has not been validated by CLSI.

#### 11.4.1 Test Procedure

Follow the general test procedure in Section 10 with the following exceptions:

1. Use the direct colony suspension procedure as described in Section 8.2 when testing streptococci. Using colonies taken directly from an overnight (18- to 20-hour) sheep blood agar culture plate, prepare a suspension equivalent to that of the 0.5 McFarland standard in either MHB or saline. Use the suspension for inoculation within 15 minutes after adjusting the turbidity.

2. Incubate the trays in ambient air at 35 ± 2 °C for 20 to 24 hours before reading the MICs.

3. Inducible clindamycin resistance can be identified in beta-hemolytic streptococci using the method described in Section 13.
11.4.2 MIC Interpretive Criteria

The antimicrobial agents suggested for routine testing of pneumococci and other streptococci are indicated in M100 Table 1A. Specific MIC interpretive criteria used when testing *Streptococcus pneumoniae* and other streptococci are listed in M100 Tables 2G, and 2H-1 and 2H-2, respectively.

12 Organisms Requiring Special Consideration

This section discusses organism groups or particular resistance mechanisms for which there are significant testing issues. Testing issues regarding both dilution and disk diffusion testing are discussed here.

12.1 Staphylococci

12.1.1 Penicillin Resistance and β-lactamase

Most staphylococci are resistant to penicillin, and penicillin is rarely an option for treatment of staphylococcal infections. Penicillin-resistant strains of *S. aureus* produce β-lactamase, and testing penicillin instead of ampicillin is preferred. Penicillin should be used to test the susceptibility of all staphylococci to all penicillinase-labile penicillins, such as ampicillin, amoxicillin, azlocillin, carbenicillin, mezlocillin, piperacillin, and ticarcillin.

An induced β-lactamase test should be performed on isolates of *S. aureus* with penicillin MICs ≤ 0.12 µg/mL or zone diameters ≥ 29 mm before reporting the isolate as penicillin susceptible. Induction can be easily accomplished by testing the growth from the zone margins surrounding an oxacillin or cefoxitin disk test. For laboratories using MIC testing, the disk for induction can be placed on a blood agar plate.

A positive β-lactamase test predicts resistance to penicillin, ampicillin, amoxicillin, azlocillin, carbenicillin, mezlocillin, piperacillin, and ticarcillin. Regardless of the penicillin MIC or zone diameter, oxacillin-resistant staphylococci should always be reported as penicillin resistant if penicillin is reported (see below).

12.1.2 Methicillin/Oxacillin Resistance

12.1.2.1 Background

Historically, resistance to the antistaphylococcal, penicillinase-stable penicillins (eg, methicillin, nafcillin, and oxacillin) has been referred to as “methicillin resistance,” and the acronyms “MRSA” (for methicillin-resistant *S. aureus*) or “MRS” (for methicillin-resistant staphylococci) are still commonly used, even though methicillin is no longer the agent of choice for testing or treatment. In this document, resistance to these agents may be referred to using several terms (eg, “MRS,” “methicillin resistance,” or “oxacillin resistance”). Most resistance to oxacillin in staphylococci is mediated by the mecA gene, which directs the production of a supplemental penicillin-binding protein, PBP 2a, and is expressed either homogeneously or heterogeneously. Homogeneous resistance is easily detected with standard testing methods, whereas heterogeneous expression may be more difficult to detect with some methods, because only a fraction of the population (eg, 1 in 100 000 cells) expresses the resistance phenotype. In the past, the presence of resistance to other classes of agents suggested an isolate was oxacillin resistant. However, some MRSA, such as those found in community-associated infections, may not be multidrug resistant (MDR).
12.1.2.2 Organism Groups

*Staphylococcus lugdunensis* are now grouped with *S. aureus* when determining methicillin/oxacillin resistance. Most *S. lugdunensis* are β-lactamase negative and nearly all are oxacillin susceptible. Oxacillin-susceptible, *mecA*-negative strains exhibit oxacillin MICs in the range of 0.25 µg/mL to 1 µg/mL, whereas *mecA*-positive strains usually exhibit MICs ≥ 4 µg/mL, characteristics more like *S. aureus* than other coagulase-negative staphylococci. Therefore, the presence of *mecA*-mediated resistance in *S. lugdunensis* is detected more accurately using the *S. aureus* interpretive criteria than the criteria for coagulase-negative staphylococci. *S. lugdunensis* should be assumed to be included with *S. aureus* in this section and in M100 Table 2C. Oxacillin and cefoxitin testing methods for coagulase-negative staphylococci exclude *S. lugdunensis*.

12.1.2.3 Methods for Detection of Oxacillin Resistance

Either oxacillin- or cefoxitin-based methods can be used for detection of *mecA*-mediated resistance in staphylococci. Oxacillin disk diffusion methods should not be used for *S. lugdunensis* and other coagulase-negative staphylococci. Cefoxitin-based methods predict the presence of *mecA*-mediated resistance only; their use is preferred to tests using oxacillin, because they are better predictors of the presence of *mecA* than are oxacillin-based methods, including the oxacillin-salt agar screening plate. Because of the rare occurrence of oxacillin resistance mechanisms other than *mecA* in *S. aureus*, some *S. aureus* may be encountered that are oxacillin resistant but *mecA* negative; these generally test as cefoxitin susceptible.

- All methods require the use of the direct colony suspension method for the preparation of inoculum (see Section 8.2).
- Incubate tests to detect MRS for a full 24 hours at 35 ± 2 °C when using oxacillin (testing at temperatures above 35 °C may not detect MRS, especially when using oxacillin) before reporting as susceptible. Incubate tests using cefoxitin for 16 to 20 hours for *S. aureus* and *S. lugdunensis* and 24 hours for coagulase negative staphylococci.
- For the most current recommendations regarding testing and reporting, refer to M100 Table 2C.

12.1.2.4 Oxacillin-Based Methods

- Of the penicillinase-stable penicillins, oxacillin is preferred for *in vitro* testing. Oxacillin is more resistant to degradation in storage and is more likely to detect heteroresistant staphylococcal strains. Cloxacillin should not be used, because it may not detect oxacillin-resistant *S. aureus*. Oxacillin susceptibility test results can be applied to the other penicillinase-stable penicillins (eg, cloxacillin, dicloxacillin, flucloxacillin, methicillin, and nafcillin).
- The addition of NaCl (2% w/v; 0.34 mol/L) is required for both agar and broth dilution testing of oxacillin to improve the detection of heteroresistant MRSA. For disk diffusion testing, MHA should not be supplemented.
- With disk diffusion testing, if an oxacillin disk is used, examine the zone of inhibition around the oxacillin disk for light growth using transmitted light (plate held up to light); any discernable growth within the zone of inhibition of oxacillin is indicative of oxacillin resistance.
- If oxacillin-intermediate results (disk diffusion testing) are obtained for *S. aureus*, perform testing for *mecA* or PBP 2a, the cefoxitin MIC or cefoxitin disk test, an oxacillin MIC test, or the oxacillin-salt agar screening test. Report the result of the alternative test rather than the oxacillin intermediate result (see below for reporting oxacillin when using cefoxitin as a surrogate test).
12.1.2.5 Cefoxitin-Based Methods

- The results of tests using cefoxitin (either broth microdilution or disk diffusion tests using a 30-µg cefoxitin disk) and alternate breakpoints (see M100 Table 2C) can be used to predict mecA-mediated oxacillin resistance in *S. aureus*. Cefoxitin tests are equivalent to oxacillin MIC tests in sensitivity and specificity for *S. aureus*.

- For coagulase-negative staphylococci, currently only the cefoxitin disk diffusion test has been validated for prediction of mecA-mediated resistance. The cefoxitin disk diffusion test has equivalent sensitivity to oxacillin MIC tests but greater specificity (ie, the cefoxitin disk test more accurately identifies oxacillin-susceptible strains than the oxacillin MIC test). There are no oxacillin disk diffusion recommendations for coagulase-negative staphylococci.

- For both *S. aureus* and coagulase-negative staphylococci, the cefoxitin disk test is easier to read than the oxacillin disk test; thus, cefoxitin is the preferred disk when performing disk diffusion.

- For *S. lugdunensis*, for disk diffusion testing, only the cefoxitin disk test should be used.

- For all staphylococci, read the zone of inhibition around the cefoxitin disk using reflected light.

- Cefoxitin is used as a surrogate for detecting oxacillin resistance. Based on the cefoxitin result, report oxacillin as susceptible or resistant.

12.1.2.6 Molecular Detection Methods

Tests for the mecA gene or the protein produced by mecA, the penicillin-binding protein 2a (PBP2a, also called PBP2′), are the most accurate methods for prediction of resistance to oxacillin.

12.1.2.7 Reporting

- Resistance may be reported any time growth is observed after a minimum of 16 hours incubation.

- If a cefoxitin-based test is used, cefoxitin is used as a surrogate for detecting oxacillin resistance. Based on the cefoxitin result, report oxacillin as susceptible or resistant.

- Report isolates of staphylococci that carry mecA, or that produce PBP 2a, the mecA gene product, as oxacillin resistant. Report isolates that do not carry mecA or do not produce PBP 2a as oxacillin susceptible if oxacillin MICs are ≤ 2 µg/mL. Because of the rare occurrence of resistance mechanisms other than mecA, report isolates that are negative for the mecA gene or do not produce PBP 2a, but for which oxacillin MICs are ≥ 4 µg/mL as oxacillin resistant.

- Oxacillin MIC interpretive criteria for coagulase-negative staphylococci correlate with the presence or absence of the gene encoding mecA for *Staphylococcus epidermidis*. These interpretive criteria may overcall resistance for other coagulase-negative staphylococci (eg, *S. saprophyticus*). For coagulase-negative staphylococci other than *S. epidermidis*, testing for mecA or PBP2a may be appropriate for strains for which the oxacillin MICs are 0.5 µg/mL to 2.0 µg/mL. For coagulase negative staphylococci, the cefoxitin disk test is preferred (see Section 12.1.2.5).

- Report oxacillin-resistant staphylococci as resistant to all other penicillins, carbapenems, cephalosporins, and β-lactam/β-lactamase inhibitors, regardless of in vitro test results with those agents. This recommendation is based on the fact that most cases of documented MRS infections have responded poorly to β-lactam therapy, or because convincing clinical data have yet to be presented that document clinical efficacy for those agents in MRS infections.
• For oxacillin-susceptible strains, report results for cephems, β-lactam/β-lactamase inhibitor combinations, and carbapenems, if tested, according to the results generated using routine interpretive criteria.

12.1.3 Vancomycin Resistance in *S. aureus*

In 2006 (M100-S16), the interpretive criteria for vancomycin and *S. aureus* were lowered to ≤ 2 µg/mL for susceptible, 4 µg/mL to 8 µg/mL for intermediate, and ≥ 16 µg/mL for resistant. For coagulase-negative staphylococci, they remain at ≤ 4 µg/mL for susceptible, 8 µg/mL to 16 µg/mL for intermediate, and ≥ 32 µg/mL for resistant.

The first occurrence of a strain of *S. aureus* with reduced susceptibility to vancomycin (MICs 4 µg/mL to 16 µg/mL) was reported from Japan in 1997, followed by reports from the United States and France. The exact mechanisms of resistance that result in elevated MICs are unknown, although they likely involve alterations in the cell wall and changes in several metabolic pathways. To date, most vancomycin-intermediate *S. aureus* (VISA) strains appear to have developed from MRSA.

From 2002 to 2007, *S. aureus* strains for which the vancomycin MICs ranged from 32 µg/mL to 1024 µg/mL have been reported in the United States. All of these strains contained a vanA gene similar to that found in enterococci. These strains are reliably detected by the broth microdilution reference method, the disk diffusion method, and the vancomycin agar screen test (see below) when the tests are incubated for a full 24 hours at 35 ± 2 ºC.

12.1.3.1 Methods for Detection of Reduced Susceptibility to Vancomycin

*S. aureus* with vancomycin MICs ≥ 32 µg/mL can be detected by either MIC, disk diffusion, or the vancomycin agar screen test. In order to recognize strains of staphylococci for which the vancomycin MICs are 4 µg/mL to 16 µg/mL, MIC testing must be performed and the tests incubated for a full 24 hours at 35 ± 2 ºC. Strains with vancomycin MICs < 32 µg/mL are not detected by disk diffusion, even with 24-hour incubation. The vancomycin agar screen test may be used to detect isolates of *S. aureus* with vancomycin MICs ≥ 8 µg/mL; however, this medium does not consistently detect *S. aureus* with vancomycin MICs of 4 µg/mL.

<table>
<thead>
<tr>
<th>Vancomycin MIC (µg/mL)</th>
<th>MIC Method</th>
<th>Disk Diffusion Method</th>
<th>Vancomycin Agar Screen</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 2 (S)</td>
<td>yes</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>4 (I)</td>
<td>yes</td>
<td>no</td>
<td>variable</td>
</tr>
<tr>
<td>8 (I)</td>
<td>yes</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>16 (R)</td>
<td>yes</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>≥ 32 (R)</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
</tbody>
</table>

*Strains of *S. aureus* for which the vancomycin zone diameter is ≥ 7 mm may have MICs from ≤ 2 µg/mL to 16 µg/mL. If disk diffusion testing is performed, the identification of isolates showing no zone of inhibition should be confirmed. Isolates of *S. aureus* producing vancomycin zones of ≥ 7 mm should not be reported as susceptible without performing a vancomycin MIC test.

Until further data on the prevalence or clinical significance of isolates with reduced susceptibility to vancomycin are known, laboratories may choose to examine MRSA strains more carefully for elevated MICs to vancomycin.
12.1.3.2 Vancomycin Agar Screen

Perform the test using the following procedure by inoculating an isolate of *S. aureus* onto Brain Heart Infusion (BHI) agar that has been supplemented with 6 μg/mL of vancomycin.

1. Prepare a direct colony suspension equivalent to a 0.5 McFarland standard as is done for MIC or disk diffusion testing (see Section 8.2).

2. Use a micropipette to deliver a 10-μL drop to the agar surface. (Alternatively, use a swab from which the excess liquid has been expressed as for the disk diffusion test and spot an area at least 10 mm to 15 mm in diameter.)

3. Incubate the plate at 35 ± 2 °C in ambient air for a full 24 hours.

4. Examine the plate carefully, using transmitted light, for evidence of small colonies (> 1 colony) or a film of growth. Greater than 1 colony or a film of growth suggests reduced susceptibility to vancomycin.

5. Confirm results for *S. aureus* that grow on the BHI vancomycin agar screen by repeating identification tests and performing vancomycin MIC tests using a CLSI reference dilution method or other validated MIC method.

6. For QC, use:
   - *E. faecalis* ATCC® 29212 or *S. aureus* ATCC® 29213 (vancomycin susceptible) – negative control. (Do not use *S. aureus* ATCC® 25923 as a negative control, as it may give false-positive results.)
   - *E. faecalis* ATCC® 51299 (vancomycin resistant) – positive control.

7. Do not reuse plates after incubation.

Currently, there are insufficient data to recommend using this agar screen test for coagulase-negative staphylococci.

12.1.3.3 Heteroresistant Vancomycin-Intermediate *Staphylococcus aureus* (hVISA)°

When first described in 1997, hVISA isolates were those *S. aureus* that contained subpopulations of cells (typically 1 in every 100 000 to 1 000 000 cells) for which the vancomycin MICs were 8 μg/mL to 16 μg/mL, ie, in the intermediate range. Because a standard broth microdilution test uses an inoculum of 5 x 10⁵ CFU/mL, these resistant subpopulations go undetected and the vancomycin MICs determined for such isolates are in the susceptible range (formerly between 1 μg/mL and 4 μg/mL). Many physicians and microbiologists initially were skeptical that heteroresistance would result in clinical treatment failures with vancomycin, because such strains were susceptible to vancomycin by the standard CLSI broth microdilution reference method. However, after reviewing both clinical and laboratory data, CLSI lowered the intermediate breakpoint for vancomycin (for *S. aureus* isolates only, not coagulase-negative staphylococci) from 8 μg/mL to 4 μg/mL, and the resistant breakpoint from ≥ 32 μg/mL to ≥ 16 μg/mL to make the breakpoints more predictive of clinical outcome. Thus, the vancomycin-susceptible breakpoint for *S. aureus* now is ≤ 2 μg/mL; the intermediate range is 4 μg/mL to 8 μg/mL, and the resistant range is ≥ 16 μg/mL. This captures many of the hVISA strains for which the vancomycin MICs are 4 μg/mL, which previously would have been designated as susceptible. Yet, some *S. aureus* strains for which the vancomycin MICs are 1 μg/mL to 2 μg/mL may still be hVISAs.
Determining the population analysis profiles of *S. aureus* isolates (ie, plating a range of dilutions of a standard inoculum of *S. aureus* [10^1 to 10^8 CFU] on a series of agar plates containing a range of vancomycin concentrations, plotting the population curve, dividing the bacterial counts by the area under the curve, and comparing the ratio derived to *S. aureus* control strains Mu3 and Mu50) has became the *de facto* “gold standard” for investigating the clinical relevance of hVISA strains in several large surveillance studies. This technique, however, is labor intensive and not suitable for routine clinical laboratories. Unfortunately, there is no standardized technique at this time that is convenient and reliable for detecting hVISA strains. The inability of both automated and standard reference susceptibility testing methods to detect the hVISA phenotype makes it difficult to identify those infections that may not respond to vancomycin therapy. Thus, confirming the presence of a heterogeneously resistant strain of *S. aureus* remains a difficult challenge.

12.1.3.4 Reporting

Vancomycin-susceptible staphylococci should be reported following the laboratory’s routine reporting protocols. For strains determined to be vancomycin nonsusceptible (ie, those with MICs ≥ 4 µg/mL and/or growth on BHI vancomycin screen agar), preliminary results should be reported following routine reporting protocols; final results should be reported after confirmation by a reference laboratory.

12.1.4 Inducible Clindamycin Resistance

Inducible clindamycin resistance can be identified using the method described in Section 13 and CLSI document M02-A (see Section 12).

12.1.5 Mupirocin Resistance

Rates of high-level mupirocin resistance may increase (ie, MICs ≥ 512 µg/mL) in *S. aureus* and is associated with the presence of the plasmid-mediated *mupA* gene. High-level mupirocin resistance can be detected using either routine disk diffusion or broth microdilution tests. For disk diffusion using a 200-µg mupirocin disk, incubate the test a full 24 hours and read carefully for any haze or growth using transmitted light. No zone of inhibition = the presence of high-level mupirocin resistance; any zone of inhibition = the absence of high-level resistance. In a recent study, the majority of *mupA*-negative isolates demonstrated mupirocin 200-µg zone diameters > 18 mm. For broth microdilution testing, an MIC of ≥ 512 µg/mL = high-level mupirocin resistance; MICs ≤ 256 µg/mL = the absence of high-level resistance. For dilution testing, a single well containing 256 µg/mL of mupirocin may be tested. For the one-concentration test, growth = high-level mupirocin resistance; no growth = the absence of high-level resistance.

12.2 Enterococci

12.2.1 Penicillin/Ampicillin Resistance

Enterococci may be resistant to penicillin and ampicillin because of production of low-affinity, penicillin-binding proteins (PBPs), or, rarely, because of the production of β-lactamase. Either the agar or broth dilution test accurately detects isolates with altered PBPs, but does not reliably detect isolates that produce β-lactamase. The rare β-lactamase-producing strains of enterococci are detected best by using a direct, nitrocefin-based, β-lactamase test (see Section 14.2). A positive β-lactamase test predicts resistance to penicillin, and amino-, carboxy-, and ureidopenicillins. Strains of enterococci with ampicillin and penicillin MICs ≥ 16 µg/mL are categorized as resistant. However, enterococci with low levels of penicillin (MICs ≤ 64 µg/mL) or ampicillin (MICs ≤ 32 µg/mL) resistance may be susceptible to synergistic killing by these penicillins in combination with gentamicin or streptomycin (in the absence of high-level resistance to gentamicin or streptomycin) if high doses of the penicillin are used. Enterococci possessing higher levels of penicillin (MICs ≥ 128 µg/mL) or ampicillin (MICs ≥ 64 µg/mL) resistance
Physicians’ requests to determine the actual MIC of penicillin or ampicillin for blood and CSF isolates of enterococci should be considered.

12.2.2 Vancomycin Resistance

Accurate detection of VRE by the agar or broth dilution test requires incubation for a full 24 hours (rather than 16 to 20 hours) before reporting as susceptible and careful examination of the plates, tubes, or wells for evidence of faint growth. A vancomycin agar screen test may also be used, as described below and in M1007 Appendix D.

12.2.3 Vancomycin Agar Screen

The vancomycin agar screening-plate procedure can be used in addition to the dilution methods described above for the detection of VRE. Perform the test using the following procedure by inoculating an enterococcal isolate onto BHI agar that has been supplemented with 6 μg vancomycin/mL.34

(1) Prepare a direct colony suspension equivalent to a 0.5 McFarland standard as is done for MIC or disk diffusion testing.

(2) Inoculate the plate using either a 1- to 10-μL loop or a swab.

   (a) Using a loop, spread the inoculum in an area 10 mm to 15 mm in diameter.

   (b) Using a swab, express as for the disk diffusion test and then spot an area at least 10 mm to 15 mm in diameter.

(3) Incubate the plate at 35 ± 2 °C in ambient air for a full 24 hours and examine carefully for evidence of growth, including small colonies (> 1 colony) or a film of growth, indicating vancomycin resistance (also see M1007 Appendix D).

(4) For QC, use:
   - *E. faecalis* ATCC® 29212 (vancomycin susceptible) – negative control;
   - *E. faecalis* ATCC® 51299 (vancomycin resistant) – positive control.

(5) Do not reuse plates after incubation.

12.2.4 High-Level Aminoglycoside Resistance

High-level resistance to gentamicin and/or streptomycin indicates that an enterococcal isolate will not be killed by the synergistic action of a penicillin or glycopeptide combined with that aminoglycoside.32 Agar or broth high-concentration gentamicin (500 μg/mL) and streptomycin (1000 μg/mL with broth microdilution; 2000 μg/mL with agar) tests can be used to screen for this type of resistance (see M1007 Appendix D). Quality control of these tests is also explained in M1007 Appendix D. Other aminoglycosides need not be tested, because their activities against enterococci are not superior to gentamicin or streptomycin.
12.3 β-Lactamase-Mediated Resistance in Gram-Negative Bacilli

12.3.1 Background

The major mechanism of resistance to β-lactam antimicrobial agents in gram-negative bacilli is production of β-lactamase enzymes. Many different types of enzymes have been reported. They may be classified as molecular Class A, B, C, or D enzymes.\(^{35}\)

<table>
<thead>
<tr>
<th>Class</th>
<th>Active site</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Serine</td>
<td>TEM-1, SHV-1, KPCs and most ESBLs including CTX-M-1</td>
</tr>
<tr>
<td>B</td>
<td>Zinc</td>
<td>Metalloenzymes; VIM, IMP, SPM</td>
</tr>
<tr>
<td>C</td>
<td>Serine</td>
<td>AmpC</td>
</tr>
<tr>
<td>D</td>
<td>Serine</td>
<td>OXA</td>
</tr>
</tbody>
</table>

12.3.2 Extended-Spectrum β-Lactamases and Plasmid-Encoded AmpC Enzymes

Extended-spectrum β-lactamases (ESBLs) are enzymes that arise by mutations in genes for common plasmid-encoded β-lactamases, such as TEM-1, SHV-1, and OXA-10; or may be only distantly related to a native enzyme, as in the case of the CTX-M β-lactamases. A similar native enzyme (OXY or K1) in *Klebsiella oxytoca* acts as an ESBL when overexpressed. ESBLs may confer resistance to penicillins, cephalosporins, and aztreonam in clinical isolates of *Klebsiella pneumoniae*, *K. oxytoca*, *E. coli*, *Proteus mirabilis*,\(^{36}\) and other genera of the family *Enterobacteriaceae*.\(^{35}\)

Plasmid-encoded AmpC-like enzymes have a similar profile to ESBLs in that they confer reduced susceptibility to penicillins, cephalosporins, and aztreonam, but also cephamycins. Because they are carried on plasmids they can be transmissible among bacteria. Although plasmid-mediated AmpC enzymes evolved from native chromosomal enzymes in other bacteria (see Section 12.3.4.1), they are found primarily in *K. pneumoniae* and *E. coli*. In contrast to the ESBLs, they are not inhibited by clavulanic acid.

Some *Enterobacteriaceae* containing ESBLs or AmpC-like enzymes will show MICs for certain extended-spectrum cephalosporins or aztreonam above those of the normal susceptible population, but below susceptibility breakpoints established prior to the emergence of ESBLs in the 1980s. M100\(^{7}\) Appendix A describes a test that can be used to screen for ESBLs in *E. coli*, *K. pneumoniae*, *K. oxytoca*, and *P. mirabilis*.

In nearly all strains expressing ESBLs of the TEM, SHV, CTX-M, and OXY type, the MICs for one or more of the extended-spectrum cephalosporins or aztreonam should decrease in the presence of clavulanic acid (see M100\(^{7}\) Appendix A for methods). Strains with plasmid-encoded AmpC-like β-lactamases will not demonstrate lowering of the MIC when combined with clavulanic acid and so will be positive on the initial screen test, but negative on the phenotypic confirmation test. At present, there are no validated phenotypic tests to confirm the presence of plasmid-encoded AmpC β-lactamases. Strains carrying both ESBLs and plasmid-encoded AmpC β-lactamases are common in some geographical regions, and their performance in the screening and confirmation tests is unpredictable. The subcommittee believes that revising susceptibility breakpoints for drugs affected by these combinations of enzymes is ultimately the best approach for providing guidance for treatment of strains with combinations of these enzymes; new revised breakpoints are expected to be published in the next one to two years. For current recommendations regarding testing and reporting, see M100\(^{7}\) Table 1 and Appendix A.
12.3.3 *Klebsiella pneumoniae* Carbapenemases

For current recommendations regarding testing and reporting of carbapenem-resistant *Enterobacteriaceae*, refer to M100\(^7\) Tables 1 and 2A and Appendix G.

12.3.4 Other β-Lactamase-Mediated Resistance in Gram-Negative Bacilli

12.3.4.1 AmpC β-lactamases in *Enterobacter*, *Citrobacter*, and *Serratia* species

The AmpC β-lactamases are chromosomal enzymes found in *Enterobacter*, *Citrobacter*, *Serratia*, and some other gram-negative species; they are resistant to the current β-lactamase inhibitors used clinically. AmpC enzymes are usually expressed in low amounts but can be induced to produce higher amounts by penicillins, carbapenems, and some cephems such as cefoxitin. The expanded-spectrum cephalosporins (cephalosporin subclasses 3 and 4) do not induce AmpC enzymes, but can be slowly hydrolyzed by them, although cefepime is particularly resistant to AmpC hydrolysis; however, they may select for stably derepressed mutants, which can emerge during therapy. AmpC producers test as resistant to cefoxitin and cefotetan. In addition, they may test resistant to carbapenems if accompanied by a porin mutation.

12.3.4.2 Metallo-β-lactamases

Metallo-β-lactamases are carbapenemases that require zinc for activity and are inhibited by substances such as ethylenediaminetetraacetic acid (EDTA) that bind zinc. *Stenotrophomonas maltophilia*, *Bacillus anthracis*, and some strains of *Bacteroides fragilis* produce a chromosomal metallo-β-lactamase. Other metalloenzymes may be carried on mobile genetic elements and can occur in *Acinetobacter* spp., *P. aeruginosa*, *Serratia marcescens*, and *K. pneumoniae*.

12.4 *Streptococcus pneumoniae*

12.4.1 Penicillin and Third-Generation Cephalosporin Resistance

Penicillin and cefotaxime or ceftriaxone or meropenem should be tested by a reliable MIC method and reported routinely with CSF isolates of *S. pneumoniae*. Such isolates should also be tested against vancomycin using the MIC or disk method. Consult M100\(^7\) Table 2G for reporting of penicillins and third-generation cephalosporins, because there are specific interpretive criteria that must be used depending on the site of infection and the penicillin formulation used for therapy. In M100\(^7\) Table 2G, breakpoints are listed for intravenous penicillin therapy for meningitis and for nonmeningitis infections. Separate breakpoints are included also for therapy of less severe infections with oral penicillin.

Amoxicillin, ampicillin, cefepime, cefotaxime, ceftriaxone, cefuroxime, ertapenem, imipenem, and meropenem may be used to treat pneumococcal infections; however, reliable disk diffusion susceptibility tests with these agents do not yet exist. Their *in vitro* activity is best determined using an MIC method.

13 Inducible Clindamycin Resistance

Macrolide-resistant isolates of *S. aureus*, coagulase-negative *Staphylococcus* spp., and beta-hemolytic streptococci may express constitutive or inducible resistance to clindamycin (methylation of the 23S rRNA encoded by the *erm* gene also referred to as MLS\(_B\) [macrolide, lincosamide, and type B streptogramin] resistance) or may be resistant only to macrolides (efflux mechanism encoded by the *msrA* gene in staphylococci or a *mef* gene in streptococci). Inducible clindamycin resistance can be detected using a disk diffusion test with clindamycin and erythromycin disks placed in close proximity\(^{10,11}\) for all staphylococci and beta-hemolytic streptococci or as a single well test using broth microdilution for staphylococci.
Using disk diffusion, the test may be done on a standard blood agar plate used for the inoculum purity check by placing a 2-µg clindamycin disk either 15 mm away (for staphylococci) or 12 mm away (for streptococci) from the edge of a 15-µg erythromycin disk or by using the standard disk diffusion procedure (see CLSI document M02, 3 Section 12). Flattening of the clindamycin zone adjacent to the erythromycin disk (referred to as a D-zone) indicates inducible clindamycin resistance. Following incubation, organisms that do not show flattening of the clindamycin zone adjacent to the erythromycin disk should be reported as tested (ie, susceptible or intermediate to clindamycin).

For staphylococci, inducible clindamycin resistance can also be detected with the broth microdilution test using the combination of erythromycin 4 µg/mL and clindamycin 0.5 µg/mL together in a single well of a broth microdilution panel. The broth microdilution test applies only to isolates that are erythromycin resistant (MICs ≥ 8 µg/mL) and clindamycin susceptible or intermediate (MICs ≤ 2 µg/mL); for these isolates, growth in the well indicates the presence of inducible clindamycin resistance. Following incubation, organisms that do not grow in the broth microdilution well should be reported as tested (ie, susceptible or intermediate to clindamycin).

Organisms that grow in the microdilution well or that show flattening of the clindamycin zone adjacent to the erythromycin disk (referred to as a D-zone) have inducible clindamycin resistance. Report such isolates as “clindamycin resistant.” Organisms that show hazy growth throughout the zone of inhibition around the clindamycin disk should be reported as clindamycin resistant whether or not they show a D-zone. The following comment may be included in patient reports for isolates that show inducible clindamycin resistance:

“This isolate is presumed to be resistant based on detection of inducible clindamycin resistance. Clindamycin may still be effective in some patients.”

Recommendations for QC and the most recent updates for testing are given in M100 7 Tables 2C, 2H-1, 2H-2, 4, and 4A, and Appendixes B and C.

14 β-Lactamase Tests

14.1 Purpose

A rapid β-lactamase test may yield clinically relevant results earlier than an MIC test with Haemophilus spp., N. gonorrhoeae, and Moraxella catarrhalis; a β-lactamase test is the only reliable test for detecting β-lactamase-producing Enterococcus spp. β-lactamase testing may also clarify the susceptibility test results of staphylococci to penicillin determined by broth microdilution, especially in strains with borderline MICs (0.06 µg/mL to 0.25 µg/mL).

A positive β-lactamase test result predicts the following:

- resistance to penicillin, ampicillin, and amoxicillin among Haemophilus spp., N. gonorrhoeae, and M. catarrhalis; and

- resistance to penicillin, and amino-, carboxy-, and ureidopenicillins among staphylococci and enterococci.

A negative β-lactamase test result does not rule out resistance due to other mechanisms. Do not use β-lactamase tests for members of the Enterobacteriaceae, Pseudomonas spp., and other aerobic, gram-negative bacilli, because the results may not be predictive of susceptibility to the β-lactams most often used for therapy.
14.2 Selecting a β-Lactamase Test

Nitrocefin-based tests are the preferred method for testing Haemophilus spp., N. gonorrhoeae, M. catarrhalis, staphylococci, and enterococci.37 Acidimetric β-lactamase tests have generally produced acceptable results with Haemophilus spp., N. gonorrhoeae, and staphylococci. Iodometric tests may be used for testing N. gonorrhoeae, but only nitrocefin-based tests should be used to test M. catarrhalis.38 Accurate detection of β-lactamase in staphylococci may require induction of the enzyme and incubation of a nitrocefin-based test for up to one hour. Induction can be easily accomplished by testing the growth from the zone margin surrounding an oxacillin or cefoxitin disk test. Care must be exercised when using these assays to ensure accurate results, including testing of known positive and negative control strains at the time clinical isolates are examined (see the manufacturer’s recommendations).

15 Reporting of MIC Results

The MIC results determined as described in this document may be reported directly to clinicians for patient-care purposes. However, it is essential for an understanding of the data by all clinicians that an interpretive category result also is provided routinely. Recommended interpretive categories for various MIC values are included in tables for each organism group and are based on evaluation data as described in CLSI document M23.2 For most agents, these categories are developed by determining MIC population distributions of a large number of isolates, including those with known mechanisms of resistance relevant to the particular class of drug. Second, the MICs are analyzed in relation to the pharmacokinetics of the drug from normal dosing regimens. Finally, when feasible, the tentative in vitro interpretive criteria are analyzed in relation to studies of clinical efficacy and microbiologic eradication in the treatment of specific pathogens as outlined in CLSI document M23.2

See Section 4.1 for definitions of the interpretive categories susceptible, intermediate, resistant, and nonsusceptible.

16 Quality Control and Quality Assurance Procedures

16.1 Purpose

In antimicrobial susceptibility testing, QC includes the procedures to monitor the performance of a test system to ensure reliable results. This is achieved by, but not limited to, the testing of QC strains with known susceptibility to the antimicrobial agents tested. The goals of a QC program are to assist in monitoring the following:

- the precision (repeatability) and accuracy of susceptibility test procedures;
- the performance of reagents used in the tests; and
- the performance of persons who carry out the tests and read the results.

A comprehensive quality assurance (QA) program helps to ensure that testing materials and processes consistently provide quality results. Quality assurance includes, but is not limited to, monitoring, evaluating, taking corrective actions (if necessary), recordkeeping, calibration and maintenance of equipment, proficiency testing, training, and QC.

16.2 Quality Control Responsibilities

Modern laboratories rely heavily on pharmaceutical and diagnostic product manufacturers for provision of reagents, media, or test systems for the performance of antimicrobial susceptibility tests. Although this
section is intended to apply to only the standard reference methods, it may be applicable to certain commercially available test systems that are based primarily, or in part, on these methods.

Manufacturers and users of antimicrobial susceptibility tests have a shared responsibility for quality. The primary purpose of QC testing performed by manufacturers (in-house reference methods or commercial methods) is to ensure that the test has been appropriately manufactured. The primary purpose of QC testing performed by laboratories (users) is to ensure that the tests are maintained and performed appropriately.

A logical division of responsibility and accountability may be described as follows:

• Manufacturers (in-house or commercial products):
  – antimicrobial stability;
  – antimicrobial labeling;
  – potency of antimicrobial stock solutions;
  – compliance with good manufacturing practices (eg, Quality System Standards);
  – integrity of product; and
  – accountability and traceability to consignee.

• Laboratories (users):
  – storage under the environmental conditions recommended by the manufacturer (to prevent drug deterioration);
  – proficiency of personnel performing tests; and
  – use of current CLSI standards (or manufacturer’s instructions for use) and adherence to the established procedure (eg, inoculum preparation, incubation conditions, interpretation of end points).

Manufacturers should design and recommend a QC program that allows users to evaluate those variables (eg, inoculum density, storage/shipping conditions) that most likely could cause user performance problems and to determine that the test is performing correctly when used according to established protocols.

16.3 Selection of Quality Control Strains for Quality Control and Quality Assurance

Use of carefully selected QC strains allows the microbiologist to have confidence that the test is performing within acceptable standards, and thus that the test results are likely to be reliable.

Each QC strain should be obtained from a recognized source (eg, the American Type Culture Collection [ATCC®]). All CLSI-recommended QC strains appropriate for the antimicrobial agent and reference method should be evaluated and expected results established according to the procedures described in CLSI document M23. Users of commercial systems should follow the QC recommendations in their instructions for use.

• Ideal strains for QC of dilution tests have MICs that fall near the middle of the concentration range tested for all antimicrobial agents (eg, an ideal QC strain would be inhibited at the fourth dilution of a seven-dilution series, but strains with MICs at either the third or fifth dilution would also be acceptable). In certain instances with newer, more potent antimicrobial agents, it may be necessary to test additional QC strains not normally tested in order to provide on-scale values.

• When three or fewer adjacent doubling dilutions of an antimicrobial agent are tested by dilution methods, QC procedures must be modified.
One possible alternative is to use one control organism with a modal MIC that is equal to or no less than one doubling dilution of the lower concentration and a second control organism with a modal MIC that is equal to, or no greater than, one doubling dilution of the higher concentration. The combination of results derived from testing these two strains should provide for at least one on-scale end point.

Another strategy is to selectively test the most labile agents included in the panels (eg, clavulanic acid combinations, imipenem, and cefaclor).

Quality control strains and their characteristics are described in Appendix D. Some of these are listed as “quality control strains” and others as “supplemental quality control strains.” These can be defined as follows:

Quality control strains are tested regularly (eg, daily or weekly) to ensure the test system is working and produces results that fall within specified limits listed in M100. The QC strains recommended in this document should be included if a laboratory performs CLSI reference MIC testing as described herein. For commercial test systems, manufacturer's recommendations should be followed for all QC procedures.

Supplemental QC strains are used to assess a particular characteristic of a test or test system in select situations or may represent alternative QC strains. For example, *Haemophilus influenzae* ATCC® 10211 is more fastidious than *H. influenzae* ATCC® 49247 or *H. influenzae* ATCC® 49766 and is used to ensure HTM can adequately support the growth of clinical isolates of *Haemophilus influenzae* and *Haemophilus parainfluenzae*. Supplemental QC strains may possess susceptibility or resistance characteristics specific for one or more special tests listed in M02 and M07. They can be used to assess a new test, for training new personnel, for competency assessment, etc. It is not necessary to include supplemental QC strains in routine daily or weekly AST QC programs.

Expected QC results for individual antimicrobial agents and tests are listed in M100 Tables 4 through 4D.

### 16.4 Storing and Testing Quality Control Strains

- Test the QC strains by standard dilution test procedures described herein using the same materials and methods that are used to test clinical isolates.

- Proper organism storage and maintenance is required to ensure acceptable performance of QC strains (also refer to Appendix E).
  - For prolonged storage, maintain stock cultures at −20 °C or below (preferably at ≤−60 °C or below or in liquid nitrogen) in a suitable stabilizer (eg, 50% fetal calf serum in broth, 10% to 15% glycerol in tryptic soy broth, defibrinated sheep blood, or skim milk) or in a freeze-dried state without significant risk of altering their antimicrobial susceptibility.

  - Subculture frozen or freeze-dried stock cultures onto appropriate media (eg, tryptic soy or blood agar for nonfastidious strains or enriched chocolate or blood agar for fastidious strains) and incubate under the appropriate conditions for the organism (primary subculture). Subculture frozen or lyophilized cultures twice before use in testing. The second subculture is referred to as Day 1 working culture.

  - Store subcultures at 2 °C to 8 °C or as appropriate for the organism type.

  - Prepare working cultures by subculturing the QC strains onto agar plates to obtain isolated colonies for testing. Prepare a new working culture each day.
- Prepare a new subculture each week to create working cultures (e.g., prepare working cultures from the same subculture for up to seven days; then prepare a new subculture on Day 8).

- Prepare new primary subcultures at least monthly from frozen, freeze-dried, or commercial cultures (e.g., subculture each week for no more than three successive weeks). For best results, some strains may require preparation of new subcultures more frequently (e.g., every two weeks).

- If an unexplained result suggests a change in the organism’s inherent susceptibility, prepare a new primary subculture, working culture, or obtain a fresh stock culture of the QC strain. See Section 16.9 for additional guidance.

### 16.5 Batch or Lot Quality Control

1. Test each new batch or lot of macrodilution tubes, microdilution trays, or agar dilution plates with the appropriate QC strains to determine if MICs obtained with the batch or lot fall within the expected range (see M1007 Table 4); if they do not, the batch or lot must be rejected.

2. Incubate at least one uninoculated tube, microdilution tray, or agar plate from each batch overnight to verify sterility of the medium.

3. New lots of MHB used to prepare macrodilution tubes or microdilution trays may need to be tested for acceptable cation content. For MHB, determine the MIC of gentamicin for *P. aeruginosa* ATCC® 27853 and compare it with the expected range (see M1007 Table 4). If the MIC is low, the broth may need to be supplemented with cations as directed in Section 10.1. When daptomycin is tested, levels of calcium cations in broth should be 50 mg/L. Mueller-Hinton agar may also be tested for appropriate cation content by disk diffusion with *P. aeruginosa* ATCC® 27853, using a 10-μg gentamicin disk, and with *S. aureus* ATCC® 25923, using a 30-μg daptomycin disk as described in CLSI document M02. To be acceptable, the inhibition zone diameter must fall within the specified gentamicin or daptomycin control range as defined in M1007.

4. Records should be kept of the lot numbers of all materials and reagents used in performing susceptibility tests.

5. *H. influenzae* ATCC® 10211 is recommended as a useful additional QC strain to verify the growth promotion properties of HTM. In particular, manufacturers of HTM are encouraged to use *H. influenzae* ATCC® 10211 as a QC test strain.

### 16.6 MIC Quality Control Limits

Acceptable MIC QC limits for a single QC test (single-drug/single-organism combination) are listed in M1007 Tables 4 through 4D.

### 16.7 Frequency of Quality Control Testing (also refer to Appendix A)

Monitor the overall performance of the test system using the QC limits by testing the appropriate QC strains each day the test is performed or, if satisfactory performance is documented (see Section 16.7.2.1), for testing the QC strains weekly. The weekly QC testing option outlined below is not applicable when MIC tests are performed less than once a week. Quality control testing should be performed each test day for MIC tests performed less than once a week.
16.7.1 Daily Testing

Performance is satisfactory for daily QC testing when no more than 3 out of 30 results obtained on consecutive test days for each antimicrobial agent/organism combination are outside the acceptable limit stated in M100\textsuperscript{7} Tables 4 through 4D. Corrective action by the laboratory is required when this frequency is exceeded (see Section 16.9).

16.7.2 Weekly Testing

16.7.2.1 Demonstrating Satisfactory Performance for Conversion From Daily to Weekly Quality Control Testing

- Test all applicable QC strains for 20 or 30 consecutive test days and document results.
- To convert from daily to weekly QC testing, no more than 1 out of 20 or 3 out of 30 MICs for each antimicrobial agent/organism combination may be outside the acceptable MIC limits stated in M100\textsuperscript{7} Tables 4 through 4D.

16.7.2.2 Implementing Weekly Quality Control Testing

- Weekly QC testing may be performed once satisfactory performance has been documented (see Section 16.7.2.1).
- Perform QC testing once per week and whenever any reagent component of the test (eg, a new lot of broth from the same manufacturer) is changed.
- If any of the weekly QC results are out of the acceptable range, corrective action is required (see Section 16.9).
- Refer to M100\textsuperscript{7} Table 4E for guidance on QC frequency with new materials or test modifications.
- These guidelines can also be used for testing systems in which an MIC is determined using three or fewer adjacent doubling dilutions of an antimicrobial agent.
- For some drugs, QC records may indicate the need for testing to be done more frequently than once a week, because of the relatively rapid degradation of the drug (see Sections 9.2.2 and 10.4.1 and M100\textsuperscript{7} Tables 4 through 4D).

16.8 Frequency of Quality Control Testing for Screening Tests

The frequency of QC screening tests is as follows:

- Quality control of vancomycin and high-level aminoglycoside screen plates may be performed weekly if these tests are routinely used (ie, at least once a week) in the laboratory, and criteria for converting from daily to weekly testing have been met.
- Quality control of screening tests may be needed each day of testing if the test is not performed routinely (at least once a week) or if the antimicrobial agent is labile (eg, oxacillin agar screen for \textit{S. aureus}).
16.9 Corrective Action

16.9.1 Out-of-Control Result Due to Identifiable Errors

If the cause of out-of-control results can be identified, correct the issue, document the reason, and retest the strain on the day the error is observed. If the repeated result is within range, no further corrective action is required. If a problem with the QC strain is suspected or identified, obtain a new working culture or subculture and retest as soon as possible.

The Troubleshooting Guide in M100 Table 4F provides guidance for troubleshooting and corrective action for out-of-range QC. Causes for the out-of-control results may include, but are not limited to:

- QC strain
  - use of the wrong QC strain;
  - improper storage;
  - inadequate maintenance (eg, use of the same working culture for > 1 month);
  - contamination;
  - nonviability; and
  - changes in the organism (eg, mutation, loss of plasmid).

- Testing supplies
  - improper storage or shipping conditions;
  - contamination;
  - inadequate volume of broth in tubes or wells;
  - use of damaged (eg, cracked, leaking) panels, plates, cards, tubes; and
  - use of expired materials.

- Testing process
  - use of the wrong incubation temperature or conditions;
  - inoculum suspensions incorrectly prepared or adjusted;
  - inoculum prepared from a plate incubated for the incorrect length of time;
  - inoculum prepared from differential or selective media containing anti-infective agents or other growth-inhibiting compounds;
  - use of wrong reagents, ancillary supplies;
  - incorrect reading or interpretation of test results; and
  - transcription error.

- Equipment
  - not functioning properly or out of calibration (eg, pipettes).

16.9.2 Out-of-Control Result With No Error Identified

If the reason for the out-of-control result cannot be identified, corrective action is required as follows.

16.9.2.1 Immediate Corrective Action

- Test the out-of-control antimicrobial agent/organism combination on the day the error is observed and/or as soon as a new working culture or subculture is available. Monitor for a total of five consecutive test days. Document all results.
  - If all five MICs for the antimicrobial agent/organism combination are within the acceptable ranges, as defined in M100 Table 4, no additional corrective action is necessary.
If any of the five MICs are still outside the acceptable range, additional corrective action is required (see Section 16.9.2.2 and M1007 Tables 4 through 4D).

- Daily control tests must be continued until final resolution of the problem is achieved.

16.9.2.2 Additional Corrective Action

When immediate corrective action does not resolve the problem, the problem is likely due to a system rather than a random error. Additional investigation and corrective action is required. Refer to Section 16.9.1 and M1007 Table 4F, Troubleshooting Guide for assistance.

If necessary, obtain a new QC strain (either from freezer storage or a reliable source) and new lots of materials (including new turbidity standards), possibly from different manufacturers. If the problem appears to be related to a manufacturer, contact and provide the manufacturer with the test results. It may also be helpful to exchange QC strains and materials with another laboratory using the same method in order to determine the root cause of unexplained system problems. Until the problem is resolved, it may be necessary to use an alternate test method.

If a problem is identified and is corrected, documentation of satisfactory performance for another five days is required to return to weekly QC testing. If a problem is not identified but results go back into control without any specific corrective action, documentation of satisfactory performance for another 20 or 30 consecutive days is required in order to return to weekly QC testing (see Section 16.7.2.1).

16.10 Reporting Patient Results When Out-of-Control Tests Occur

Whenever an out-of-control result occurs or corrective action is necessary, careful assessment of whether to report patient test results should be made on an individual patient basis, taking into account if the source of the error, when known, is likely to have affected relevant patient test results. Considerations may include, but are not limited to:

- Size and direction of error (eg, slightly or significantly increased MIC/zone size, slightly or significantly decreased MIC/zone size).
- Is the patient result close to the interpretive breakpoint?
- Results with other QC organisms.
- Results with other antimicrobial agents.
- Is the QC strain/antimicrobial agent an indicator for a procedural or storage issue (eg, inoculum dependent, heat labile)? Refer to M1007 Table 4F, Troubleshooting Guide.

Options to consider include suppressing the results for an individual antimicrobial agent; retrospectively reviewing individual patient or cumulative data for unusual patterns; and using an alternate test method or a reference laboratory until the problem is resolved.

16.11 Verification of Patient Test Results

Multiple test parameters are monitored by following the QC recommendations described in this standard. However, acceptable results derived from testing QC strains do not guarantee accurate results when testing patient isolates. It is important to review all of the results obtained from all drugs tested on a patient’s isolate prior to reporting the results. This should include, but not be limited to ensuring that:
the antimicrobial susceptibility results are consistent with the identification of the isolate;

the results from individual agents within a specific drug class follow the established hierarchy of activity rules (eg, third-generation cephalosporins are more active than first- or second-generation cephalosporins against *Enterobacteriaceae*); and

the isolate is susceptible to those agents for which resistance has not been documented (eg, vancomycin and *Streptococcus* spp.) and for which only “susceptible” interpretive criteria exist in M100.7

Unusual or inconsistent results should be verified by checking for the following:

previous results on the patient (eg, Did the patient have the same isolate with an unusual antibiogram previously?);

previous QC performance (Is there a similar trend or observation with recent QC testing?); and

problems with the testing supplies, process, or equipment (see Section 16.9.1 and M1007 Table 4F, Troubleshooting Guide).

If a reason for the unusual or inconsistent result cannot be ascertained, a repeat of the susceptibility test or the identification or both is in order. Sometimes, it is helpful to use an alternative test method for the repeat test. A suggested list of results that may require verification is included in M1007 Appendix E. Each laboratory must develop its own policy for verification of unusual or inconsistent antimicrobial susceptibility test results. This policy should emphasize those results that may significantly impact patient care.

### 16.12 Other Control Procedures

#### 16.12.1 Growth Control

Each microdilution broth tray, macrodilution broth series, and agar dilution plate series should include a growth control of basal medium without antimicrobial agent to assess viability of the test organisms. With the broth tests, the growth control also serves as a turbidity control for reading end points.

#### 16.12.2 Purity Control

Following inoculation of agar or broth dilution tests, streak a sample from each inoculum on a suitable agar plate and incubate overnight to detect mixed cultures and to provide freshly isolated colonies in case retesting proves necessary. This step is particularly important for broth dilutions where mixed cultures are likely to go unrecognized.

#### 16.12.3 Inoculum Control

Perform QC plate counts at least quarterly with representative inocula to ensure that the 0.5 McFarland standard and the procedures for standardizing and diluting inocula remain under control. To do this, remove samples for plate counts immediately after inoculation from the growth-control well of microdilution trays, the growth-control tube of a macrodilution series, or in agar dilution, a random reservoir well of the replicator seed block (see Section 10.5).
16.12.4 End-point Interpretation Control

Monitor end-point interpretation periodically to minimize variation in the interpretation of MIC end points among observers. All laboratory personnel who perform these tests should independently read a selected set of dilution tests. Record the results and compare to the results obtained by an experienced reader. All readers should agree within ±1 twofold concentration increment of one another.39

17 Limitations of Dilution Test Methods

17.1 Application to Various Organism Groups

The dilution methods described in this document are standardized for testing rapidly growing pathogens, which include *Staphylococcus* spp., *Enterococcus* spp., the *Enterobacteriaceae*, *P. aeruginosa*, *Pseudomonas* spp., *Acinetobacter* spp., *Burkholderia cepacia*, and *S. maltophilia*, and they have been modified for testing some fastidious organisms, such as *Haemophilus* spp. (M1007 Table 2E), *N. gonorrhoeae* (M1007 Table 2F), *N. meningitidis* (M1007 Table 2J), and streptococci (M1007 Tables 2G, 2H-1, and 2H-2). For additional guidance on standardized susceptibility testing of organisms not presently included in M023 or M07, please refer to CLSI document M45.5

17.2 Misleading Results

Dangerously misleading results can occur when certain antimicrobial agents are tested and reported as susceptible against specific organisms. These combinations include, but may not be limited to, the following:

- first- and second-generation cephalosporins, cephemycins, and aminoglycosides against *Salmonella* and *Shigella* spp.;
- penicillins, β-lactam/β-lactamase inhibitor combinations, cephems, and carbapenems against oxacillin-resistant *Staphylococcus* spp.;
- aminoglycosides (except high concentrations), cephalosporins, clindamycin, and trimethoprim-sulfamethoxazole against *Enterococcus* spp.; and
- penicillins, cephalosporins, and aztreonam against ESBL-producing *K. pneumoniae*, *K oxytoca*, *E. coli*, and *P. mirabilis*.

17.3 Emergence of Resistance

Some antimicrobial agents are associated with the emergence of resistance during prolonged therapy. Therefore, isolates that are initially susceptible may become resistant within three to four days after the initiation of therapy. This occurs most frequently in *Enterobacter*, *Citrobacter*, and *Serratia* spp. with third-generation cephalosporins; in *P. aeruginosa* and *Acinetobacter* spp. with all antimicrobial agents; and in staphylococci with quinolones and vancomycin (VISAs).

In certain circumstances, repeat testing to detect emerging resistance might be warranted earlier than within three to four days, and the decision to do so requires knowledge of the specific situation and the severity of the patient’s condition. Laboratory guidelines on when to perform repeat susceptibility testing should be determined after consultation with the medical staff.
18 Screening Tests

Screening tests for oxacillin-resistant *S. aureus* and high-level aminoglycoside-resistant enterococci have been shown to be comparable in reliability to standard methods for detecting clinically significant resistance; additional confirmatory tests are unnecessary. Limitations of other screening tests (eg, for vancomycin resistance in enterococci [M1007 Appendix D] and *S. aureus* [M1007 Appendix B] and ESBLs in certain *Enterobacteriaceae* [M1007 Appendix A]) and the necessity for further confirmation are given in the individual tables.
References


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Appendix A. Quality Control Protocol Flow Charts

Quality Control Protocol: Each Test Day

QC each test day (Section 16.7.1)

≤ 3 out of 30 results out of range
- QC each test day until weekly criteria are met – see next page

> 3 out of 30 results out of range
- Corrective Action (Section 16.9)
- Error identified and corrected
  - Retest
  - Results in range – continue QC each test day
  - All results in range
    - Continue QC each test day
    - Investigate possible source of errors
  - Any results out of range
    - Additional Corrective Action (Section 16.9.2.2)

No error identified
- Immediate Corrective Action (Section 16.9.2.1)
  - Retest and monitor for 5 consecutive test days
Appendix A. (Continued)

Quality Control Protocol: Weekly Testing

Demonstrate Satisfactory Performance (Section 16.7.2.1)

≤ 1 out of 20 or ≤ 3 out of 30 results out of range

Implement weekly QC testing

Any result out of range

Corrective Action (Section 16.9)

Error identified and corrected

Retest

Results in range

Return to weekly QC testing

Results out of range

Immediate Corrective Action (Section 16.9.2.1)

No error identified

Retest and monitor for 5 consecutive test days

All results in range

Return to weekly QC testing

Any results out of range

Additional Corrective Action (Section 16.9.2.2)

Investigate possible source of errors

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Appendix B. Preparation of Supplements, Media, and Reagents

B1. Supplements

B1.1 Cation Stock Solutions

(1) To prepare a magnesium stock solution, dissolve 8.36 g of MgCl\textsubscript{2}•6H\textsubscript{2}O in 100 mL of deionized water. This solution contains 10 mg of Mg\textsuperscript{++}/mL.

(2) To prepare a calcium stock solution, dissolve 3.68 g of CaCl\textsubscript{2}•2H\textsubscript{2}O in 100 mL of deionized water. This solution contains 10 mg of Ca\textsuperscript{++}/mL.

(3) Sterilize by membrane filtration and store at 2 ºC to 8 ºC.

B1.2 Lysed Horse Blood (50%)

(1) Aseptically mix equal volumes of defibrinated horse blood and sterile, deionized water (now 50% LHB).

(2) Freeze and thaw the 50% horse blood until the cells are thoroughly lysed (about five to seven freeze-thaw cycles).

(3) For use in broth dilution tests, the combination of broth and LHB must be clear, and this can be accomplished by centrifuging the 50% LHB at 12,000 \textit{x} g for 20 minutes. Decant the supernatant and recentrifuge if necessary.

(4) Aseptically add appropriate amounts of the 50% LHB to the CAMHB to yield a final concentration of 2.5% to 5% LHB.

(5) Check the pH after aseptic addition of the blood to the autoclaved and cooled broth.

(6) The blood may be added to microdilution trays either when they are first dispensed or after they are thawed just prior to inoculation. If added after plate preparation, the blood must be added along with the inoculum so further dilution of the antimicrobial agent in the tray does not occur (see Section 10.4.2), and the final concentration of LHB in the well is 2.5% to 5%. Alternatively, add 5 µL of 50% LHB to each 100-µL well prior to tray inoculation. This is only acceptable if total volume of liquids added to the well does not exceed 10 µL.

(7) The 50% LHB can be stored frozen at ≤−20 ºC indefinitely.

**NOTE:** Lysed horse blood prepared for use in broth microdilution is available from commercial sources.

B2. Agar Media

B2.1 Mueller-Hinton Agar

Use MHA from a commercially available dehydrated base. Only MHA formulations that have been tested according to, and that meet the acceptance limits described in, CLSI document M06\textsuperscript{1} should be used.

(1) Prepare according to the manufacturer’s directions.
Appendix B. (Continued)

(2) For use in preparation of agar dilution plates, immediately after autoclaving, allow the agar to cool to 45 °C to 50 °C in a water bath before aseptically adding antimicrobial solutions and heat-labile supplements, and pouring the plates (see Section 9.2.2).

(3) Check the pH of each batch of MHA when the medium is prepared. The exact method used depends largely on the type of equipment available in the laboratory. The agar medium should have a pH between 7.2 and 7.4 at room temperature and must therefore be checked after gelling. If the pH is less than 7.2, certain drugs will appear to lose potency (e.g., aminoglycosides and macrolides), while other agents may appear to have excessive activity (e.g., tetracyclines). If the pH is greater than 7.4, the opposite effects can be expected. Check the pH by one of the following means:

- Macerate enough agar to submerge the tip of a pH electrode.
- Allow a small amount of agar to solidify around the tip of a pH electrode in a beaker or cup.
- Use a surface electrode.

(4) Do not add supplemental calcium or magnesium cations to MHA.

B2.2 Mueller-Hinton Agar + 2% NaCl

(1) Follow instructions in Section B2.1 except that for oxacillin, methicillin, or nafcillin testing of staphylococci, add 20 g NaCl to 1 L of MHA (2% w/v) before autoclaving.

B2.3 Mueller-Hinton Agar With 5% Sheep Blood or 2.5% to 5% Lysed Horse Blood

(1) Prepare MHA as described above through Section B2.1 (2). When it has cooled to 45 °C to 50 °C, add 50 mL of defibrinated sheep blood or 25 mL to 50 mL of LHB to 1 L of MHA.

(2) Check the pH after aseptic addition of the blood to the autoclaved and cooled medium. The final pH should be the same as unsupplemented MHA, pH 7.2 to 7.4.

(3) NOTE: For testing *H. pylori*, the sheep blood should be ≥ 2 weeks old before using to prepare the agar medium.

B2.4 GC Agar + 1% Defined Growth Supplement

(1) Use a 1% Defined Growth Supplement that contains the following ingredients per liter:

- 1.1 g L-cystine;
- 0.03 g guanine HCl;
- 0.003 g thiamine HCl;
- 0.013 g *p*-aminobenzoic acid;
- 0.01 g vitamin B12;
- 0.1 g thiamine pyrophosphate (cocarboxylase);
- 0.25 g nicotinamide adenine dinucleotide (NAD);
- 1 g adenine;
- 10 g L-glutamine;
- 100 g glucose;
- 0.02 g ferric nitrate; and
- 25.9 g L-cysteine HCl.
Appendix B. (Continued)

(2) Prepare GC agar base from a commercially available dehydrated base according to the manufacturer’s instructions.

(3) After autoclaving, cool to 45 °C to 50 °C in a 45 °C to 50 °C water bath.

(4) Add 10 mL of 1% Defined Growth Supplement.

B3. Broth Media

B3.1 Cation-Adjusted Mueller-Hinton Broth

Obtain dehydrated MHB base from a commercially available source that is available in either of the following two forms:

- containing the desired concentration of cations (20 mg to 25 mg of Ca ++/L and 10 mg to 12.5 mg of Mg ++/L); or

- without supplemental cation, in which case, the cation content is usually inadequate.

The first does not require further cation adjustment (except for testing daptomycin when the broth must be supplemented to 50 mg/L of Ca ++), if prepared according to the manufacturer’s recommendations.

For the second, the amount of Ca ++ and Mg ++ cations present in the medium must be determined before it is prepared. This information is usually available in the Certificate of Analysis for the lot of medium obtained; if not, contact the manufacturer for that information. The concentration of cations already present in the broth must be accounted for when calculating the amount of Ca ++ or Mg ++, if any, that needs to be added.

The instructions for cation adjustments below should be followed only when the initial MHB has been certified by the manufacturer or measured by the user to contain no or inadequate amounts of Ca ++ and Mg ++ when assayed for total divalent cation content by atomic absorption spectrophotometry. An inadequate cation content may lead to erroneous results. For testing daptomycin, MHB must be supplemented to 50 mg/L of Ca ++.

(1) Prepare MHB according to the manufacturer’s recommendations, autoclave, and prior to adding supplemental cations, chill overnight at 2 °C to 8 °C (or in an ice bath if broth is to be used the same day).

(2) With stirring, add 0.1 mL of chilled Ca ++ or Mg ++ stock solution per liter of broth for each desired increment of 1 mg/L in the final concentration in the adjusted MHB. It is preferable to add the Mg ++ before adding the Ca ++. This medium is called cation-adjusted MHB (CAMHB).

Example:

For MHB that contains:

- 4.3 mg/L Ca ++ and
- 3.8 mg/L Mg ++
Appendix B. (Continued)

(a) For Ca++, the final amount needed is 20 mg/L to 25 mg/L.

\[ \text{Final amount needed} - \text{Amount in medium} = \text{Amount to be added} \]

\[ 25 \text{ mg/L} - 4.3 \text{ mg/L} = 20.7 \text{ mg/L} \]

The medium needs 20.7 mg/L added.

\[ 20.7 \text{ mg/L} \times 0.1 \text{ mL} = 2.07 \text{ mL} \]  
[0.1 mL for each 1 mg/L needed]

Therefore, add 2.07 mL of Ca++ stock per liter.

NOTE: For testing daptomycin, supplement the broth to 50 mg/L of Ca++.

(b) For Mg++, the final amount needed is 10 mg/L to 12.5 mg/L.

\[ 12.5 \text{ mg/L} - 3.8 \text{ mg/L} = 8.7 \text{ mg/L} \]

The medium needs 8.7 mg/L.

\[ 8.7 \text{ mg/L} \times 0.1 \text{ mL} = 0.87 \text{ mL} \]  
[0.1 mL for each 1 mg/L needed]

Therefore, add 0.87 mL of Mg++ stock per liter.

(3) Check the pH after additions of the cations. The final pH should be 7.2 to 7.4.

B3.2 Cation-Adjusted Mueller-Hinton Broth + 2% NaCl

(1) Follow instructions in Section B3.1 except that for oxacillin, methicillin, or nafcillin testing of staphylococci, add 20 g NaCl to 1 L of MHB (2% w/v) before autoclaving.

B3.3 Cation-Adjusted Mueller-Hinton Broth + 2.5% to 5% Lysed Horse Blood

(1) Prepare CAMHB as described in Section B3.1, except for each liter of broth desired, prepare with 50 mL to 100 mL less deionized water to account for the addition of the blood.

(2) After autoclaving and cooling, add 50 mL to 100 mL 50% LHB to 1 L of broth (see Section B1.2 for preparation of LHB).

(3) Check the pH after aseptic addition of the blood to the medium. The final pH should be the same as CAMHB, pH 7.2 to 7.4.

B3.4 Cation-Adjusted Mueller-Hinton Broth + 0.002% Polysorbate 80 (P-80)

(1) Prepare a 2% solution of P-80 by diluting full-strength P-80 50-fold (ie, 1 mL P-80 + 49 mL of deionized water).

(2) Add 1 mL of the 2% P-80 solution per liter of broth before autoclaving.

(3) The final pH should be the same as CAMHB, pH 7.2 to 7.4.
Appendix B. (Continued)

B3.5  *Haemophilus* Test Medium Broth

In its broth form, HTM consists of the following ingredients:

- MHB;
- 15 μg/mL β-NAD;
- 15 μg/mL bovine or porcine hematin;
- 5 g/L yeast extract; and
- 0.2 IU/mL thymidine phosphorylase (if sulfonamides or trimethoprim are to be tested).

1. Prepare a fresh hematin stock solution by dissolving 50 mg of hematin powder in 100 mL of 0.01 mol/L NaOH with heat and stirring until the powder is thoroughly dissolved.

2. Prepare an NAD stock solution by dissolving 50 mg of NAD in 10 mL of distilled water; filter sterilize.

3. Prepare MHB from a commercially available dehydrated base according to the manufacturer’s directions, adding 5 g of yeast extract and 30 mL of hematin stock solution to 1 L of MHB.

4. After autoclaving, cool to 45 ºC to 50 ºC; aseptically add cations, if needed as in CAMHB.

5. If sulfonamides or trimethoprim are to be tested, add 0.2 IU thymidine phosphorylase to the medium aseptically.

6. Aseptically add 3 mL of the NAD stock solution.

7. The pH should be 7.2 to 7.4.

NOTE: *H. influenzae* (ATCC® 10211) is recommended as a useful additional QC strain to verify the growth promotion properties of HTM. In particular, manufacturers of HTM are encouraged to use *H. influenzae* ATCC® 10211 as a supplemental QC test strain.

B4.  Reagents

B4.1  0.5 McFarland Turbidity Standard

1. Prepare a 0.048 mol/L BaCl₂ (1.175% w/v BaCl₂•2H₂O) stock solution.

2. Prepare a 0.18 mol/L (0.36 N) H₂SO₄ (1% v/v) stock solution.

3. With constant stirring to maintain a suspension, add 0.5 mL of the BaCl₂ solution to 99.5 mL of the H₂SO₄ stock solution.

4. Verify the correct density of the turbidity standard by measuring absorbance using a spectrophotometer with a 1-cm light path and matched cuvettes. The absorbance at 625 nm should be 0.08 to 0.13 for the 0.5 McFarland standard.

5. Transfer the barium sulfate suspension in 4- to 6-mL aliquots into screw-cap tubes of the same size as those used for standardizing the bacterial inoculum.

6. Tightly seal the tubes and store in the dark at room temperature.
Appendix B. (Continued)

(7) Vigorously agitate the barium sulfate turbidity standard on a vortex mixer before each use and inspect for a uniformly turbid appearance. Replace the standard if large particles appear. Mix latex particle suspensions by inverting gently, not on a vortex mixer.

(8) The barium sulfate standards should be replaced or their densities verified monthly.

NOTE: McFarland standards made from latex particle suspension are available commercially. When used, they should be mixed by inverting gently (not on a vortex mixer) immediately prior to use.

Reference for Appendix B

Appendix C. Conditions for Dilution Antimicrobial Susceptibility Tests

C1. Conditions for Dilution Antimicrobial Susceptibility Tests for Nonfastidious Organisms

<table>
<thead>
<tr>
<th>Organism/Organism group</th>
<th>M100 Table</th>
<th>Medium</th>
<th>Inoculum</th>
<th>Incubation</th>
<th>Incubation Time</th>
<th>Minimal Quality Control</th>
<th>Comments/Modifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entrobacteriaceae</td>
<td>2A</td>
<td>CAMHB; MHA</td>
<td>Direct colony suspension in MHB or saline or growth method</td>
<td>35 ± 2 °C; ambient air</td>
<td>16 to 20 hours</td>
<td>E. coli ATCC® 25922 E. coli ATCC® 35218 (for β-lactam/β-lactamase inhibitor combinations)</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>2B-1</td>
<td>CAMHB; MHA</td>
<td>Direct colony suspension in MHB or saline or growth method</td>
<td>35 ± 2 °C; ambient air</td>
<td>16 to 20 hours</td>
<td>E. coli ATCC® 25922 P. aeruginosa ATCC® 27853 E. coli ATCC® 35218 (for β-lactam/β-lactamase inhibitor combinations)</td>
<td></td>
</tr>
<tr>
<td>Acinetobacter spp.</td>
<td>2B-2</td>
<td>CAMHB; MHA</td>
<td>Direct colony suspension in MHB or saline or growth method</td>
<td>35 ± 2 °C; ambient air</td>
<td>20 to 24 hours</td>
<td>E. coli ATCC® 25922 P. aeruginosa ATCC® 27853 E. coli ATCC® 35218 (for β-lactam/β-lactamase inhibitor combinations)</td>
<td></td>
</tr>
<tr>
<td>Burkholderia cepacia</td>
<td>2B-3</td>
<td>CAMHB; MHA</td>
<td>Direct colony suspension in MHB or saline or growth method</td>
<td>35 ± 2 °C; ambient air</td>
<td>20 to 24 hours</td>
<td>E. coli ATCC® 25922 P. aeruginosa ATCC® 27853</td>
<td></td>
</tr>
<tr>
<td>Stenotrophomonas maltophilia</td>
<td>2B-4</td>
<td>CAMHB; MHA</td>
<td>Direct colony suspension in MHB or saline or growth method</td>
<td>35 ± 2 °C; ambient air</td>
<td>20 to 24 hours</td>
<td>E. coli ATCC® 25922 P. aeruginosa ATCC® 27853</td>
<td></td>
</tr>
<tr>
<td>Other Non-Enterobacteriaceae</td>
<td>2B-5</td>
<td>CAMHB; MHA</td>
<td>Direct colony suspension in MHB or saline or growth method</td>
<td>35 ± 2 °C; ambient air</td>
<td>16 to 20 hours</td>
<td>E. coli ATCC® 25922 P. aeruginosa ATCC® 27853 E. coli ATCC® 35218 (for β-lactam/β-lactamase inhibitor combinations)</td>
<td></td>
</tr>
<tr>
<td>Organism/Organism group</td>
<td>M100 Table</td>
<td>Medium</td>
<td>Inoculum</td>
<td>Incubation</td>
<td>Incubation Time</td>
<td>Minimal Quality Control</td>
<td>Comments/Modifications</td>
</tr>
<tr>
<td>-------------------------</td>
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<td>----------</td>
<td>------------</td>
<td>------------------</td>
<td>--------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td><strong>Staphylococcus spp.</strong></td>
<td>2C</td>
<td>CAMHB; CAMHB with 2% NaCl for oxacillin, methicillin, and nafcillin; CAMHB supplemented to 50 µg/mL calcium (daptomycin); MHA; MHA with 2% NaCl for oxacillin, methicillin, and nafcillin</td>
<td>Direct colony suspension in MHB</td>
<td>35 ± 2 °C; ambient air</td>
<td>16 to 20 hours; 24 hours for oxacillin, methicillin, nafcillin, and vancomycin</td>
<td>S. aureus ATCC® 29213 E. coli ATCC® 35218 (for β-lactam/β-lactamase inhibitor combinations)</td>
<td>Agar dilution has not been validated for daptomycin. Testing at temperatures above 35 °C may not detect MRS. Use S. aureus ATCC® BAA-977 and S. aureus ATCC® BAA-976 for supplemental QC of the clindamycin induction test.</td>
</tr>
<tr>
<td><strong>Enterococcus spp.</strong></td>
<td>2D</td>
<td>CAMHB; CAMHB supplemented to 50 µg/mL calcium (daptomycin); MHA</td>
<td>Direct colony suspension in MHB or saline or growth method</td>
<td>35 ± 2 °C; ambient air</td>
<td>16 to 20 hours; 24 hours for vancomycin</td>
<td>E. faecalis ATCC® 29212</td>
<td>Agar dilution has not been validated for daptomycin.</td>
</tr>
</tbody>
</table>
C2. Conditions for Dilution Antimicrobial Susceptibility Tests for Fastidious Organisms

<table>
<thead>
<tr>
<th>Organism/Organism group</th>
<th>M100 Table</th>
<th>Medium</th>
<th>Inoculum</th>
<th>Incubation</th>
<th>Incubation Time</th>
<th>Minimal Quality Control</th>
<th>Comments/Modifications</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. influenzae</em></td>
<td>2E</td>
<td>HTM broth</td>
<td>Direct colony suspension in MHB or saline prepared from overnight (preferably 20- to 24-hour) chocolate agar plate ( b )</td>
<td>35 ± 2 °C; ambient air</td>
<td>20 to 24 hours</td>
<td><em>H. influenzae</em> ATCC® 49247 <em>H. influenzae</em> ATCC® 49766 ( E. coli ) ATCC® 35218 (for amoxicillin-clavulanate acid)</td>
<td></td>
</tr>
<tr>
<td><em>N. gonorrhoeae</em></td>
<td>2F</td>
<td>GC agar base + 1% defined growth supplement ( d )</td>
<td>Direct colony suspension in MHB or 0.9% phosphate-buffered saline, pH 7.0, prepared from overnight chocolate agar plate incubated in 5% ( \text{CO}_2 )</td>
<td>36 ± 1 °C (do not exceed 37 °C); 5% ( \text{CO}_2 )</td>
<td>20 to 24 hours</td>
<td><em>N. gonorrhoeae</em> ATCC® 49226</td>
<td></td>
</tr>
<tr>
<td><em>S. pneumoniae</em></td>
<td>2G</td>
<td>CAMHB + 2.5% to 5% LHB</td>
<td>Direct colony suspension in MHB or saline using colonies from an overnight (18- to 20-hour) sheep blood agar plate</td>
<td>35 ± 2 °C; ambient air</td>
<td>20 to 24 hours</td>
<td><em>S. pneumoniae</em> ATCC® 49619</td>
<td></td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>2H-1 and 2H-2</td>
<td>CAMHB + 2.5% to 5% LHB; CAMHB + 2.5% to 5% LHB supplemented to 50 µg/mL calcium for daptomycin; MHA + 5% sheep blood or 2.5% to 5% LHB when testing sulfonamides</td>
<td>Direct colony suspension in MHB or saline</td>
<td>35 ± 2 °C; ambient air (CO(_2) if necessary for growth with agar dilution)</td>
<td>20 to 24 hours</td>
<td><em>S. pneumoniae</em> ATCC® 49619 Agar dilution has not been validated for daptomycin.</td>
<td></td>
</tr>
<tr>
<td><em>N. meningitidis</em></td>
<td>2L</td>
<td>CAMHB + 2.5% to 5% LHB MHA + 5% sheep blood</td>
<td>Direct colony suspension in MHB or saline prepared from overnight chocolate agar plate incubated in 5% ( \text{CO}_2 ) ( c )</td>
<td>35 ± 2 °C; 5% ( \text{CO}_2 )</td>
<td>20 to 24 hours</td>
<td><em>S. pneumoniae</em> ATCC® 49619 (ambient air or 5% ( \text{CO}_2 ); azithromycin QC test must be incubated in ambient air) <em>E. coli</em> ATCC® 25922 (ambient air or 5% ( \text{CO}_2 ); for ciprofloxacin, nalidixic acid, minocycline, and sulfisoxazole)</td>
<td></td>
</tr>
</tbody>
</table>

\( a \) 3.5 years old \( b \) Direct colony suspension in MHB or saline prepared from overnight (preferably 20- to 24-hour) chocolate agar plate incubated in 5% \( \text{CO}_2 \) \( c \) Direct colony suspension in MHB or saline prepared from overnight chocolate agar plate incubated in 5% \( \text{CO}_2 \); when using agar dilution, the plate is incubated in ambient air. \( d \) Direct colony suspension in MHB or saline prepared from overnight chocolate agar plate incubated in 5% \( \text{CO}_2 \).
Appendix C. (Continued)

a If no suspension method is indicated, then either growth or direct colony suspension can be used.
b This suspension will contain approximately 1 to 4 x 10^8 CFU/mL. Exercise care in preparing this suspension, because higher inoculum concentrations may lead to false-resistant results with some β-lactam antimicrobial agents, particularly when β-lactamase-producing strains of H. influenzae are tested.
c The use of a cysteine-free supplement is required for agar dilution tests with carbapenems and clavulanate. Cysteine-containing defined growth supplements do not significantly alter dilution test results with other drugs.
d Colonies grown on sheep blood agar may be used for inoculum preparation. However, the 0.5 McFarland suspension obtained from sheep blood agar will contain fewer CFU/mL. This must be taken into account when preparing the final dilution prior to panel inoculation, as guided by colony counts.
# Appendix D. Quality Control Strains for Antimicrobial Susceptibility Tests

(Refer to current edition of M100 for the most updated version of this table)

<table>
<thead>
<tr>
<th>Quality Control Strain</th>
<th>Organism Characteristics</th>
<th>Disk Diffusion Tests</th>
<th>MIC Tests</th>
<th>Screening Tests</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Enterococcus faecalis</em> ATCC® 29212</td>
<td>•Nonfastidious gram-positives</td>
<td></td>
<td></td>
<td>•Vancomycin agar High-level aminoglycoside resistance</td>
<td>Assess suitability of medium for sulfonamide or trimethoprim MIC tests.¹⁶</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> ATCC® 51299</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC® 25922</td>
<td>•β-lactamase negative</td>
<td>•Nonfastidious gram-negatives •<em>Neisseria meningitidis</em></td>
<td>•Nonfastidious gram-negatives •<em>Neisseria meningitidis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC® 35218</td>
<td>•Contains plasmid-encoded TEM-1 β-lactamase (non-ESBL)¹₂,¹³,¹⁴</td>
<td>•β-lactam/β-lactamase inhibitor combinations</td>
<td>•β-lactam/β-lactamase inhibitor combinations</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em> ATCC® 49247</td>
<td>•BLNAR</td>
<td>•<em>Haemophilus</em> spp.</td>
<td>•<em>Haemophilus</em> spp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em> ATCC® 700603</td>
<td>•Contains SHV-18 ESBL¹₂,¹³,¹⁴</td>
<td>•ESBL screen and confirmatory tests</td>
<td>•ESBL screen and confirmatory tests</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Neisseria gonorrhoeae</em> ATCC® 49226</td>
<td>•CMRNG, chromosomally mediated penicillin resistant</td>
<td>•<em>Neisseria gonorrhoeae</em></td>
<td>•<em>Neisseria gonorrhoeae</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> ATCC® 27853®</td>
<td>•Contains inducible AmpC β-lactamase</td>
<td>•Nonfastidious gram-negatives</td>
<td>•Nonfastidious gram-negatives •Potential agents of bioterrorism</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC® 25923</td>
<td>•β-lactamase negative •mecA negative •Little value in MIC testing due to extreme susceptibility to most drugs</td>
<td>•Nonfastidious gram-positives</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹²,¹³,¹⁴ © Clinical and Laboratory Standards Institute. All rights reserved.
<table>
<thead>
<tr>
<th>Quality Control Strain</th>
<th>Organism Characteristics</th>
<th>Disk Diffusion Tests</th>
<th>MIC Tests</th>
<th>Screening Tests</th>
<th>Other</th>
</tr>
</thead>
</table>
| *Staphylococcus aureus* ATCC® 29213 | • Weak β-lactamase producing strain  
• *mecA* negative | | • Nonfastidious gram-positives  
• Potential agents of bioterrorism | • Oxacillin agar | • Assess suitability of cation content in each batch/lot of Mueller-Hinton for daptomycin disk diffusion |
| *Staphylococcus aureus* ATCC® 43300 | • Oxacillin-resistant, *mecA* positive | • Cefoxitin disk testing | | • Cefoxitin MIC testing | • Oxacillin agar |
| *Staphylococcus aureus* ATCC® BAA-1708 | • High-level mupirocin resistance mediated by the *mupA* gene | • Screening test for high-level mupirocin resistance | | • Screening test for high-level mupirocin resistance | |
| *Streptococcus pneumoniae* ATCC® 49619 | • Penicillin intermediate by altered penicillin binding protein | • *Streptococcus pneumoniae*  
• *Streptococcus spp.*  
• *Neisseria meningitidis* | | | |
| *Klebsiella pneumoniae* ATCC® BAA-1705 | • KPC-producing strain  
• Modified Hodge test positive | • Phenotypic confirmatory test for carbapenemase production (modified Hodge test) | | | |
| *Klebsiella pneumoniae* ATCC® BAA-1706 | • Resistant to carbapenems by mechanisms other than carbapenemase  
• Modified Hodge test negative | • Phenotypic confirmatory test for carbapenemase production (modified Hodge test) | | | |
| *Staphylococcus aureus* ATCC® BAA-976 | • Contains *msrA*-mediated macrolide-only resistance | • Assess disk approximation tests with erythromycin and clindamycin (D-zone test negative) | | • QC – See M100 Appendix B | |
| *Staphylococcus aureus* ATCC® BAA-977 | • Contains inducible *ermA*-mediated resistance | • Assess disk approximation tests with erythromycin and clindamycin (D-zone test positive) | | • Routine QC for inducible clindamycin test by MIC method—See M100 Appendix B | |
Appendix D. (Continued)

Footnotes

a. *E. coli* ATCC® 35218 is recommended only as a control organism for β-lactamase inhibitor combinations, such as those containing clavulanic acid, sulbactam, or tazobactam. This strain contains a plasmid-encoded β-lactamase (non-ESBL); subsequently, the organism is resistant to many penicillinase-labile drugs but susceptible to β-lactam/β-lactamase inhibitor combinations. The plasmid must be present in the QC strain for the QC test to be valid; however, the plasmid may be lost during storage at refrigerator or freezer temperatures. To ensure the plasmid is present, test the strain with a β-lactam agent alone (either ampicillin, amoxicillin, piperacillin, or ticarcillin) in addition to a β-lactam/β-lactamase inhibitor agent (eg, amoxicillin-clavulanate). If the strain loses the plasmid, it will be susceptible to the β-lactam agent when tested alone, indicating that the QC test is invalid and a new culture of *E. coli* ATCC® 35218 must be used.

b. Careful attention to organism maintenance (eg, minimal subcultures) and storage (eg, –60 °C or below) is especially important for QC strains *E. coli* ATCC® 35218, *K. pneumoniae* ATCC® 700603, and *Klebsiella pneumoniae* ATCC® BAA-1705 because spontaneous loss of the plasmid encoding the β-lactamase or carbapenemase has been documented. Plasmid loss leads to QC results outside the acceptable limit, such as decreased MICs for *E. coli* ATCC® 35218 with enzyme-labile penicillins (eg, ampicillin, piperacillin, and ticarcillin), decreased MICs for *K. pneumoniae* ATCC® 700603 with cephalosporins and aztreonam, and false negative modified Hodge test (MHT) with *K. pneumoniae* ATCC® BAA-1705.

c. Develops resistance to β-lactam antimicrobial agents after repeated transfers onto laboratory media. Minimize by removing new culture from storage at least monthly or whenever the strain begins to show resistance.

d. End points should be easy to read (as 80% or greater reduction in growth as compared to the control) if media have acceptable levels of thymidine.


g. See Section 16.3.
Appendix E. Quality Control Strain Maintenance (also refer to Section 16.4)

1. Rehydrate new stock culture or obtain strain from frozen stock.

2. Subculture to appropriate media and incubate (primary subculture).

3. Subculture, incubate, and store as appropriate for the organism type. Use isolated colonies from Days 1 to 7 as working cultures for testing.

4. Prepare new subculture every seven days (from slant or Day 1 working culture). Store at appropriate temperature for organism type. Use fresh working cultures each test day.

5. Repeat for Week 3 and Week 4. After four weeks, discard subculture and pull strain from freezer stock or rehydrate new stock culture.

**NOTE 1:** Subculture frozen or lyophilized cultures twice before use.

**NOTE 2:** For QC testing, select isolated colonies from working cultures.

**NOTE 3:** If contaminated or questionable performance, prepare new primary subculture, working culture, or obtain new stock culture.

**NOTE 4:** It may be necessary to prepare new subcultures or Week 1/Day 1 working cultures every two weeks for some organisms (eg, *P. aeruginosa* ATCC® 27853, *E. faecalis* ATCC® 51299, *S. pneumoniae* ATCC® 49619).
Clinical and Laboratory Standards Institute consensus procedures include an appeals process that is described in detail in Section 8 of the Administrative Procedures. For further information, contact CLSI or visit our website at www.clsi.org.

Summary of Comments and Subcommittee Responses

M07-A7: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Seventh Edition

NOTE: All comments received are answered by the subcommittee and the responses published in M02 and M07. Between the three-year cycle of text revisions, any comments received are published in M100. All comments are then published in the next revisions of M02 and M07.

General

1. I am preparing to test minimal inhibitory concentration (MIC) values and had a question about the dilutions. Someone mentioned to me that it is recommended to make only four dilutions from each antibiotic and then make a new standard at a lower concentration. I cannot find reference to that in my reading of M07. Would it be possible to do serial dilutions of the antibiotics rather than the method outlined in Table 6 of M100? I am concerned about being told that serial dilutions are only good for four dilutions, because this is a standard practice I have always used to quantify CFU/mL and if it is not accurate with these antibiotic standards, then who is to say it is accurate for quantifying CFU/mL? And if it is accurate for quantifying CFU/mL, then why is it not accurate for quantifying MIC values for the antibiotics? Sorry for being confused. I have been handed a protocol already in place that seems to have a lot of unnecessary dilutions and testing being done to determine the MIC and am trying to scale it back.

   • In the experience of many of the subcommittee members who have been preparing reference dilution panels or plates for many years, they have never done what you describe, ie, prepare intermediate stock solutions when diluting more than four tubes. M07-A8 states in Section 10.4.1, “For the intermediate (10x) antimicrobial solutions, dilute the concentrated antimicrobial stock solution (see Section 7.3) as described in M100 Table 7 (previous Table 6) or by making serial twofold dilutions.”

2. In CLSI document M02, the disk diffusion zone diameters are given with equivalent MIC breakpoints. In the overwhelming majority, they correspond to the MIC breakpoints printed in M07. However, some do not (eg, gentamicin and amikacin with Enterobacteriaceae). Do you know why? Also, some of the MIC equivalent breakpoints are not in doubling dilutions (eg, in Table 2A, the Susceptible equivalent breakpoints are ≤12 µg/mL for netilmicin and ≤6 µg/mL for kanamycin). Why?

   • MIC equivalents listed in M02 represent the MIC breakpoints used when the zone size diameters were first determined. Since the M02 document was published before the M07 document, occasional discrepancies have existed and these mainly occur with the aminoglycosides. However, in M100-S19, the MIC and zone diameter interpretive criteria in all the Table 2s were combined in the same Table for each of the organism groups and the equivalent MIC breakpoints (or MIC correlates) for disk diffusion no longer appear in the Tables. A table listing the older MIC equivalents for zone diameters where discrepancies occurred between M02 and M07 is available in the minutes of the AST Subcommittee meeting of 11-13 June 2008 as Attachment 3.

3. Our pulmonologist has requested that we test Staphylococcus spp. and Enterobacteriaceae against moxifloxacin. The PharmD gave me a moxifloxacin product insert that gives different interpretive criteria (≥19 = susceptible) than those listed in M100 (≥24 mm = susceptible). Their product insert gives the same interpretive criteria for Enterobacteriaceae, and the CLSI document does not list ANY moxifloxacin interpretations for Enterobacteriaceae.

   I realize that we may use FDA or CLSI interpretive criteria, but the difference here is so great—19 mm would be RESISTANT per CLSI—that I don’t feel comfortable reporting any results until I get a satisfactory explanation.
Although there are several reasons why the CLSI and FDA moxifloxacin breakpoints for staphylococci differ, the most important point for the laboratorian to understand is that CLSI breakpoints can be used for all staphylococci including MRSA, whereas the FDA breakpoints apply only to methicillin-susceptible staphylococci (per the FDA label for clinical use of the drug), so the laboratory should not report the drug on MRSA if using the FDA breakpoints. CLSI breakpoints for testing moxifloxacin with Enterobacteriaceae have not been determined, but FDA breakpoints are available for use. It is important to note that moxifloxacin is not approved for treatment of urinary tract infections due to low urinary concentrations and, thus, should not be tested on urinary isolates. The decision regarding which drugs to report for certain organism groups and which breakpoints to use should be made by the laboratory following discussions with appropriate stakeholders such as infectious disease practitioners and the pharmacy department, as well as the Pharmacy & Therapeutics and Infection Control committees of the medical staff. Clinical laboratories may implement newly approved or revised disk CLSI breakpoints as soon as they are published in M100. If a susceptibility testing device includes antimicrobial test concentrations sufficient to allow interpretation of susceptibility to an agent using the CLSI MIC breakpoints, a laboratory could, after appropriate validation, choose to interpret and report results using CLSI breakpoints.

4. What is the recommended frequency for quality control of various agar screening tests (e.g., chromogenic media, vancomycin agar screen)?

Media containing antimicrobials used for primary isolation are not part of the scope of the susceptibility testing documents M02 and M07 (see CLSI document M22).

Single drug susceptibility tests/screens should be treated like other susceptibility tests (multiple concentrations or multiple drugs) until such time that recommendations and appropriate supportive data are available to streamline.

Tables 1 and 2B-4

In a recent College of American Pathologists (CAP) survey, participants were told that for S. maltophilia, they should have only reported results and interpretive breakpoints for the antimicrobial agents listed in Table 1. The question concerns minocycline, which is listed in the S. maltophilia column. Most laboratories can test tetracycline, but not minocycline. In footnote b in Table 1 (M100-S15), it states that tetracycline can be used to predict susceptibility (not Intermediate or Resistant) to minocycline. Is the same statement true for S. maltophilia? There are no tetracycline breakpoints listed in the draft of Table 2B-4, S. maltophilia (M100-S16). If minocycline is not available on the antimicrobial susceptibility testing medical device system the laboratory is using, and the S. maltophilia isolate is susceptible to tetracycline, should the laboratories report the tetracycline result or not?

It is true that isolates of S. maltophilia that are susceptible to tetracycline are also susceptible to minocycline and doxycycline. However, >90% of S. maltophilia strains (personal communication, R. Jones, Sentry Antimicrobial Surveillance Program) are resistant to tetracycline but susceptible to minocycline and doxycycline, so testing tetracycline as a surrogate in place of the other tetracyclines is not recommended, because the vast majority of strains would be called resistant. When testing was done to determine criteria for testing S. maltophilia and Burkholderia cepacia, the CLSI working group chose to include only agents that were active, that were recommended by experts as therapies of these infections, and for which the recommended breakpoints were proven to be reproducible.

Table 2A and 2B-5

Enterobacteriaceae and non-Enterobacteriaceae, which are resistant to tobramycin and amikacin, but susceptible to gentamicin, most likely produce a 6′-acetyltransferase. In this case, only one of the three gentamicin subcomponents, C1, remains active. Since the fraction of C1 varies between gentamicin formulations and C1 appears to have different pharmacokinetics than gentamicin as a whole (Antimicrob Agents Chemother. 1975;7:328-332), are the gentamicin interpretive breakpoints accurate in these cases? Would it be reasonable to report gentamicin susceptibility as intermediate or provide a comment that gentamicin activity is uncertain?
The commenter raises an interesting question. The subcommittee has no data that support changing the susceptible category to intermediate or resistant. However, when an isolate that is gentamicin susceptible and amikacin and tobramycin resistant is encountered and selective reporting is used by the laboratory, the susceptibility to gentamicin and the resistance to tobramycin and amikacin should all be reported.

Table 2B-2

7. I am a microbiology supervisor with a question regarding interpretations for *Acinetobacter* to tigecycline. I have an infectious disease doctor complaining that this drug has been out for over a year, and still no interpretations and guidelines regarding this drug have been published. I have the 2008 standards and see this is true. Any time frame or information that you may have so that I could pass some pertinent information on to this doctor would be appreciated.

Interpretive criteria for tigecycline are not included in the CLSI documents for any genera, because the drug manufacturer has not presented the necessary data for review by the subcommittee for subsequent publication of breakpoints in M100. In the meantime, one ordinarily could refer to the drug package insert for the US Food and Drug Administration (FDA) breakpoints; however, breakpoints for *Acinetobacter* are not included in the FDA list at this time because there is no clinical indication for tigecycline against *Acinetobacter*.

8. CLSI document M100-S17 has MIC susceptibility ranges for colistin and polymyxin B against *Acinetobacter* sp., but there are no standards listed for disk diffusion on this isolate. Our Infectious Disease staff sometimes requests that colistin and polymyxin B be tested against multidrug resistant (MDR) *Acinetobacter* isolates; and since these drugs are not available on our commercial conventional microdilution panels, I order these antimicrobials as (MIC) antibiotic gradient strips from a commercial source. The company, however, requires that a disclaimer be signed stating that we will use colistin and polymyxin B for INVESTIGATIONAL USE ONLY; a disclaimer is only good for six months and a new disclaimer must be signed for each new order. Should colistin and polymyxin B not be used for clinical purposes and are indeed for investigational use only?

There are no disk diffusion criteria for *Acinetobacter* in M100 because the disk test does not correlate with MIC tests and is therefore unreliable. Questions about the commercial gradient strip test should be addressed to the manufacturer. The use of colistin or polymyxin B for clinical treatment is a medical decision.

Table 2G

9. I have a question about reporting cefepime (meningitis) and/or cefepime (nonmeningitis). In M100-S18 Table 2G M07-MIC, cefepime (nonmeningitis) has a comment (11), “Only report interpretations for nonmeningitis and include the nonmeningitis notation on the report.” There is not a US FDA-approved indication for the use of cefepime for meningitis. Just below the cefepime (nonmeningitis) entry, cefepime (meningitis) is listed with interpretative values. When would it be appropriate to use this?

The CLSI documents are also for use outside the United States where cefepime might be used for treatment of meningitis, which is the reason those criteria are included in Table 2G. You should discuss with your Medical Director how to handle reporting of cefepime, but one solution in the United States might be to report only cefepime (nonmeningitis) with a note that cefepime is not US FDA approved for treatment of meningitis.
Summary of Delegate Comments and Subcommittee Responses

M07-A8: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Eighth Edition

Section 10.3.1(4) and 10.4.1(1), Preparing and Storing Diluted Antimicrobial Agents

1. The sentence that starts with the single pipette can be used for adding the stock solution to the first tube...The next sentence says a separate pipette should be used for each remaining dilution in the set. This sentence is often questioned by users...If you are saying to use a different pipette for each dilution it needs to say: “For each dilution in the doubling dilution series, a different pipette should be used...to perform each dilution in the series.” We get questioned on this issue a lot.

- The text was revised to clarify using a new pipette for each subsequent dilution step.

Appendix G in M100

2. This new appendix is good. I am glad to see the Hodge test described. It would be helpful to explain the difference between KPC and metallo β-lactamases a little, and point out more clearly that the Hodge will be positive for either. Many people are looking at the EDTA tests to differentiate KPC/MBL and I wonder why these are not described. Some guidance re: plasmid-mediated AmpC confirmation would be welcomed.

- Several different carbapenemases have been reported in Enterobacteriaceae. The most common types are the class A β-lactamase, KPC, and the metallo-β-lactamases (MBLs), VIM, and IMP.¹ The prevalence of bacteria expressing these enzymes varies geographically. The modified Hodge test can detect both KPC and MBL enzymes, but does not differentiate between them. The screening and confirmatory test recommendations were largely derived following testing of US isolates of Enterobacteriaceae, and provides for a high level of sensitivity (> 90%) and specificity (> 90%) in detecting KPC-type carbapenemases in these isolates. The sensitivity and specificity of the test for detecting low-level metallo-β-lactamase production is not known. MBLs can be differentiated from other carbapenemases by performing a phenotypic test that uses a chelator of Zn²⁺ to selectively inhibit the MBL.¹ Several phenotypic tests to detect MBLs have been described. Differentiation of MBLs from other carbapenemases is not important for patient management, but these tests can be useful for epidemiological studies. The subcommittee has not yet conducted any studies to address the accuracy of routine methods to detect plasmid-mediated AmpC resistance

Reference:
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 approach is based on the model presented in the most current edition of CLSI/NCCLS document HS01—A Quality Management System Model for Health Care. 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:

- Documents & Records
- Organization
- Personnel
- Equipment
- Purchasing & Inventory
- Information Management
- Process Control
- Occurrence Management
- Assessments—External & Internal
- Process Improvement
- Customer Service
- Facilities & Safety

M07-A8 addresses the QSEs 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 steps to deliver the particular product or service that the organization or entity provides. For example, CLSI/NCCLS document GP26—Application of a Quality Management System Model for Laboratory Services defines a clinical laboratory path of workflow, which consists of three sequential processes: preexamination, examination, and postexamination. All clinical laboratories follow these processes to deliver the laboratory’s services, namely quality laboratory information.

M07-A8 addresses the clinical laboratory path of workflow steps 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.
Related CLSI Reference Materials


M11-A7  Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard—Seventh Edition (2007). This document provides reference methods for the determination of minimal inhibitory concentrations (MICs) of anaerobic bacteria by agar dilution and broth microdilution.

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 Occupationaly 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.


M45-A  Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Approved Guideline (2006). This document provides guidance to clinical microbiology laboratories for standardized susceptibility testing of infrequently isolated or fastidious bacteria that are not presently included in CLSI documents M02, M07, or M11. The tabular information in this document presents the most current information for drug selection, interpretation, and quality control for the infrequently isolated or fastidious bacterial pathogens included in this guideline.

M100-S19  Performance Standards for Antimicrobial Susceptibility Testing; Nineteenth Informational Supplement (2009). This document provides updated tables for the CLSI antimicrobial susceptibility testing standards M02-A10 and M07-A8.

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

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