M02-A11
Vol. 32 No. 1
Replaces M02-A10
January 2012 Vol. 29 No. 1

# Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Eleventh Edition

This document contains the current Clinical and Laboratory Standards Institute–recommended methods for disk susceptibility testing, criteria for quality control testing, and updated tables for interpretive zone diameters.

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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M02-A11
ISBN 1-56238-781-2 (Print)
ISBN 1-56238-782-0 (Electronic)
ISSN 1558-6502 (Print)
Volume 32 Number 1 ISSN 2162-2914 (Electronic)

# Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Eleventh Edition

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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. In many clinical microbiology laboratories, an agar disk diffusion method is used routinely for testing common, rapidly growing, and certain fastidious bacterial pathogens. Clinical and Laboratory Standards Institute document M02-A11—*Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Eleventh Edition* includes a series of procedures to standardize the way disk diffusion tests are performed. The performance, applications, and limitations of the current CLSI-recommended methods are also described.

The supplemental information (M100[1] tables) presented with this standard represents the most current information for drug selection, interpretation, and quality control using the procedures standardized in M02. 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-S21[2]) appear in boldface type and are also summarized in the front of the document.

Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Eleventh Edition*. CLSI document M02-A11 (ISBN 1-56238-781-2 [Print]; ISBN 1-56238-782-0 [Electronic]). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2012.

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## Suggested Citation

CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Eleventh Edition.* CLSI document M02-A11. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.

**Proposed Standard**
July 1975
**Tentative Standard**
October 1979
**Approved Standard**
December 1984
**Tentative Standard—Fourth Edition**
November 1988
**Approved Standard—Fourth Edition**
April 1990
**Approved Standard—Fifth Edition**
December 1993
**Approved Standard—Sixth Edition**
January 1997
**Approved Standard—Seventh Edition**
January 2000
**Approved Standard—Eighth Edition**
January 2003
**Approved Standard—Ninth Edition**
January 2006
**Approved Standard—Tenth Edition**
January 2009
**Approved Standard—Eleventh Edition**
January 2012

ISBN 1-56238-781-2 (Print)
ISBN 1-56238-782-0 (Electronic)
ISSN 1558-6502 (Print)
ISSN 2162-2914 (Electronic)

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### Acknowledgment

It has been a distinct 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 working group chairholders of the Subcommittee on Antimicrobial Susceptibility Testing for their valuable contributions during the last three years. Those individuals who are not listed in the committee and working group lists in this document are as follows:

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## Foreword

In this revision of CLSI document M02, several sections were added or revised as outlined below in the Summary of Changes. The latest version of the M100[1] tables, 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.

Many other editorial and procedural changes in this edition of M02 resulted from meetings of the Subcommittee on Antimicrobial Susceptibility Testing since 2009. Specific changes for the M100[1] tables are summarized at the beginning of CLSI document M100.[1] The most important changes in M02 are summarized below.

## Summary of Major Changes in This Document

**CLSI Reference Methods vs Commercial Methods and CLSI vs US Food and Drug Administration Interpretive Criteria (Breakpoints) – Textbox**
Added the recommendation for each laboratory to check with the manufacturer of its commercial susceptibility testing device for information on the breakpoints used in its system's software.

**Section 4.1, Definitions**
Clarified the definition for nonsusceptible.
Added a definition for breakpoint/interpretive criteria.

**Section 6.2.1.2, β-Lactam/β-Lactamase Inhibitor Combinations**
Added information on the activity of β-lactamase inhibitors.

**Section 6.2.1.3, Cephems (Including Cephalosporins)**
Added information on a new subclass, cephalosporins with anti–methicillin-resistant *Staphylococcus aureus* (MRSA) activity.

**Section 6.2.2.6, Nitroimidazoles**
Added nitroimidazoles as a new section, which includes the antimicrobial agents metronidazole and tinidazole.

**Section 6.2.2.7, Oxazolidinones**
Added oxazolidinones as a new section, which includes the antimicrobial agent linezolid.

**Section 6.2.2.9, Streptogramins**
Added streptogramins as a new section, which includes the antimicrobial agents quinupristin-dalfopristin and linopristin-flopristin.

**Section 6.2.2.10, Tetracyclines**
Clarified that tigecycline is in a separate class, glycylcycline.

**Section 6.2.2.11, Single-Drug Classes**
Deleted streptogramins.
Added fusidic acid, macrocyclics, mupirocin, and spectinomycin.

**Section 6.4, Suggested Guidelines for Routine and Selective Testing and Reporting**
Added a recommendation for laboratories to develop a protocol to address isolates that are confirmed as resistant to all agents on their routine test panel.

## Summary of Major Changes in This Document (Continued)

**Section 7.1.4, Effects of Variation in Divalent Cations**
Added information on the unreliability of testing daptomycin by disk diffusion.

**Section 9.3, Reading Plates and Interpreting Results**
Added recommendations if testing cefoxitin, methicillin, or nafcillin against *Staphylococcus* spp.

**Section 10.4.2, *Streptococcus pneumoniae* Zone Diameter Interpretive Criteria**
Clarified indications of susceptibility with oxacillin for nonmeningitis isolates of *S. pneumoniae*.

**Section 11.1.1, Penicillin Resistance and β-lactamase**
Added additional recommendations for the use of nitrocefin-based tests or the penicillin disk diffusion zone-edge test for isolates of *Staphylococcus* with penicillin MICs $\leq 0.12$ µg/mL or zone diameters $\geq 29$ mm for β-lactamase production before reporting as susceptible.

**Section 11.1.2.7, Reporting**
Added information for reporting oxacillin-resistant staphylococci isolates with cephalosporins with anti-MRSA activity.

**Section 11.1.3.2, Vancomycin Agar Screen**
Added information on when variation in screen test results may occur.

**Sections 11.3, Gram-Negative Bacilli and 11.3.1, Background**
Clarified the active site for each of the molecular classes in the table.
Added OXY to Class A.
Added NDM to Class B.
Clarified the example for OXA in Class D.
Added information on β-lactamase enzymes and their resistance mechanisms.

**Section 11.3.2, Extended-Spectrum β-Lactamases (ESBLs)**
Provided additional information on ESBLs being inhibitor-susceptible enzymes.
Added information on how β-lactam interpretive breakpoints are set at MIC values to recognize ESBL activity.

**Section 11.3.3, AmpC Enzymes**
Expanded information on AmpC β-lactamases and their mode of action.

**Section 11.3.4, Carbapenemases (Carbapenem-Resistant *Enterobacteriaceae*)**
Combined previous sections on other β-lactamase-mediated resistance and metallo-β-lactamase, and provided more detailed information on carbapenemase activity in clinical isolates of *Enterobacteriaceae*, including addition of a table with examples.

**Section 12, Inducible Clindamycin Resistance**
Clarified testing method to include specific disk placement for staphylococci.

**Section 13.2, Selecting a β-Lactamase Test**
Penicillin zone-edge test method added as an alternative method for detection of β-lactamase in staphylococci.

**Section 16.2, Misleading Results**
Added cephalosporins with anti-MRSA activity to the second bullet.

## Summary of Major Changes in This Document (Continued)

**Appendix C, Conditions for Disk Diffusion Antimicrobial Susceptibility Tests**
Clarified incubation for *Staphylococcus* spp.
Added caution comment for testing *Neisseria meningitidis* isolates.

**Appendix D, Quality Control Strains for Antimicrobial Susceptibility Tests**
Added note regarding the lack of a disk diffusion test for daptomycin.

## Summary of CLSI Processes for Establishing Interpretive Criteria and Quality Control Ranges

The Clinical and Laboratory Standards Institute (CLSI) is an international, voluntary, nonprofit, interdisciplinary, standards-developing, and educational organization accredited by the American National Standards Institute, which develops and promotes the 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, QC parameters, and how the data are to be presented for evaluation are described in CLSI document M23.[3]

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. Although 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.

## CLSI Reference Methods vs Commercial Methods and CLSI vs US Food and Drug Administration Interpretive Criteria (Breakpoints)

It is important for users of M02-A11 and M07-A9 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 by clinical laboratories for routine susceptibility testing or 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 the agency has concluded 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, variations in doses used 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.[3]

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, it is acceptable for laboratories that use FDA-cleared susceptibility testing devices to use existing FDA interpretive breakpoints. Either FDA or CLSI susceptibility interpretive breakpoints are acceptable to clinical laboratory accrediting bodies in the United States. Policies in other countries may vary. Each laboratory should check with the manufacturer of its antimicrobial susceptibility test system for additional information on the interpretive criteria used in its system's software.

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 disk diffusion test breakpoints may be implemented as soon as they are published in M100.[1] 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, health care providers and educators, and pharmaceutical and diagnostic microbiology industries. Using the CLSI voluntary consensus process, the subcommittee develops standards that promote accurate antimicrobial susceptibility testing and appropriate reporting.

The mission of the Subcommittee on Antimicrobial Susceptibility Testing is to:

- Develop standard reference methods for antimicrobial susceptibility tests.
- Provide quality control parameters for standard test methods.
- Establish 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 diffusion, antibiotic, antimicrobial agents, disk diffusion, susceptibility testing

# Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Eleventh Edition

## 1 Scope

This document describes the standard agar disk diffusion techniques used to determine the *in vitro* susceptibility of bacteria that grow aerobically. The document addresses preparation of agar plates, testing conditions (including inoculum preparation and standardization, incubation time and incubation temperature), interpretation of results, quality control (QC) procedures, and limitations of disk diffusion 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 using dilution methods are found in CLSI document M07[4]; standards for testing the *in vitro* susceptibility of bacteria that grow anaerobically are found in CLSI document M11.[5] Guidelines for standardized susceptibility testing of infrequently isolated or fastidious bacteria that are not included in CLSI documents M02, M07,[4] or M11[5] are available in CLSI document M45.[6]

## 2 Introduction

A variety of laboratory methods can be used to measure the *in vitro* susceptibility of bacteria to antimicrobial agents. In many clinical microbiology laboratories, an agar disk diffusion method is used routinely for testing common, rapidly growing, and certain fastidious bacterial pathogens. This document describes the performance, applications, and limitations of the standardized disk diffusion test method. Recommendations of the International Collaborative Study[7] and regulations[8,9] proposed by the US Food and Drug Administration (FDA) have been reviewed, and appropriate sections were incorporated into this standard. Other susceptibility testing methods exist that provide essentially equivalent results to the CLSI methods described herein. The FDA is responsible for the approval of commercial devices used in the United States. CLSI does not approve or endorse commercial products or devices.

Disk diffusion tests based solely on the presence or absence of a zone of inhibition without regard to the size of the zone are not acceptable for determining antimicrobial susceptibility. Reliable results can only be obtained with disk diffusion tests that use the principle of standardized methodology and zone diameter measurements correlated with minimal inhibitory concentrations (MICs) with strains known to be susceptible or resistant to various antimicrobial agents.

The methods described herein must be followed explicitly to obtain reproducible results. The standardized method currently recommended by the CLSI Subcommittee on Antimicrobial Susceptibility Testing is based on the method originally described by Bauer et al.[10] This is the most thoroughly described disk diffusion method for which interpretive standards have been developed and supported by laboratory and clinical data.

This document describes methods, QC, and interpretive criteria recommended for disk diffusion 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 annual informational supplements (M100).[1]

## 3 Standard Precautions

Because it is often impossible to know what isolates or specimens might be infectious, all patient and laboratory specimens are treated as infectious and handled according to "standard precautions." Standard precautions are guidelines that combine the major features of "universal precautions and body substance isolation" practices. Standard precautions cover the transmission of all known infectious agents and thus

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are more comprehensive than universal precautions, which are intended to apply only to transmission of blood-borne pathogens. Standard and universal precaution guidelines are available from the Centers for Disease Control and Prevention (CDC).[11] For specific precautions for preventing the laboratory transmission of all known infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all known infectious diseases, refer to CLSI document M29.[12]

# 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 dosage recommended to treat the site of infection is used.

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

4) **nonsusceptible** – a category used for isolates for which only a susceptible interpretive criterion has been designated because of the absence or rare occurrence of resistant strains. Isolates that have minimal inhibitory concentrations (MICs) above or zone diameters below the value indicated for the susceptible breakpoint should be reported as nonsusceptible; **NOTE 1:** An isolate that is interpreted as nonsusceptible does not necessarily mean that the isolate has a resistance mechanism. It is possible that isolates with MICs above the susceptible breakpoint that lack resistance mechanisms may be encountered within the wild-type distribution subsequent to the time the susceptible-only breakpoint is set; **NOTE 2:** For strains yielding results in the "nonsusceptible" category, organism identification and antimicrobial susceptibility test results should be confirmed. (See M100[1] Appendix A.)

**breakpoint/interpretive criteria** – minimal inhibitory concentration (MIC) or zone diameter value used to indicate susceptible, intermediate, and resistant as defined above.

For example, for antimicrobial agent X with interpretive criteria of:

| | MIC (μg/mL) | Zone Diameter (mm) |
|---|---|---|
| Susceptible | ≤4 | ≥20 |
| Intermediate | 8–16 | 15–19 |
| Resistant | ≥32 | ≤14 |


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"Susceptible breakpoint" is 4 μg/mL or 20 mm.
"Resistant breakpoint" is 32 μg/mL or 14 mm.

**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.[13,14]

**quality assurance (QA)** – a part of quality management focused on providing confidence that quality requirements will be fulfilled (ISO 9000)[15]; **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 (modified from ISO 9000)[15]; **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 and Acronyms

| | |
|---|---|
| AST | antimicrobial susceptibility testing |
| ATCC | American Type Culture Collection |
| BHI | Brain Heart Infusion |
| BSC | biological safety cabinet |
| BSL | Biosafety Level (USA) |
| CDC | Centers for Disease Control and Prevention |
| CFU | colony-forming units |
| CSF | cerebrospinal fluid |
| DNA | deoxyribonucleic acid |
| EDTA | ethylenediaminetetraacetic acid |
| ESBL | extended-spectrum β-lactamase |
| FDA | US Food and Drug Administration |
| HTM | *Haemophilus* Test Medium |
| hVISA | heteroresistant vancomycin-intermediate *Staphylococcus aureus* |
| KPC | *Klebsiella pneumoniae* carbapenemase |
| MHA | Mueller-Hinton agar |
| MHB | Mueller-Hinton broth |
| MHT | modified Hodge test |
| MIC | minimal inhibitory concentration |
| MRS | methicillin-resistant staphylococci |
| MRSA | methicillin-resistant *Staphylococcus aureus* |
| NAD | nicotinamide adenine dinucleotide |
| PBP | penicillin-binding protein |
| PBP 2a | penicillin-binding protein 2a |
| QA | quality assurance |
| QC | quality control |
| RNA | ribonucleic acid |
| TEM | Temoneira (first patient from whom a TEM β-lactamase–producing strain was reported) |
| US | United States |
| VRE | vancomycin-resistant enterococci |

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# 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. 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.

# 6 Selection of Antimicrobial Agents for Routine Testing and Reporting

Selection of the most appropriate antimicrobial agents to test and 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[1] Tables 1A and 1B 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 agents in M100[1] Tables 1A and 1B are recommendations that are considered appropriate for testing and reporting. To avoid misinterpretation, routine reports to physicians should include only those antimicrobial agents appropriate for therapeutic use, as suggested in Tables 1A and 1B. 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 epidemiological information, but they should not be included on patient reports. However, such results should be available (in the laboratory) to the infection control practitioner and/or hospital epidemiologist.


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## 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 (see M100[1] Glossary I).

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

β-lactam antimicrobial agents all share the common, central, four-member β-lactam ring and 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, 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 a considerably 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 β-lactam class antimicrobial agent and a second agent that has minimal antibacterial activity, but functions as an inhibitor of some β-lactamases. β-lactamase inhibitors generally do not have antimicrobial activity on their own, but will potentiate the activity of the β-lactam antimicrobial agent combined with it. Currently, three β-lactamase inhibitors are in use: clavulanic acid, sulbactam, and tazobactam. The results of tests of only the β-lactam 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 cephalosporins; the agents in subclasses cephamycin, oxacephem, and carbacephems; as well as a new subclass, cephalosporins with anti–methicillin-resistant *Staphylococcus aureus* (MRSA) activity (see M100[1] Glossary I). Cephalosporins are often referred to as “first-,” “second-,” “third-,” or “fourth-generation” cephalosporins, 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 the penicillin class; 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.

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#### 6.2.1.5 Monobactams

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

### 6.2.2 Non-β-lactams (see M100[1] 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, 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 treatment of a gram-positive bacterial infection in the penicillin-allergic patient, and it is useful for therapy of infections due to β-lactam-resistant, gram-positive bacterial strains (eg, MRSA and some enterococci).

#### 6.2.2.4 Lipopeptides

Lipopeptides are a structurally related group of antimicrobial agents, for which the 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 physiological 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 antimicrobial agents consists of several subgroups,


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including azithromycin, clarithromycin, dirithromycin, the ketolide telithromycin, and the fluoroketolide solithromycin.

#### 6.2.2.6 Nitroimidazoles

Nitroimidazoles, which include metronidazole and tinidazole, are bactericidal agents that are converted intracellularly in susceptible organisms to metabolites that disrupt the host deoxyribonucleic acid (DNA); they are active only against strictly anaerobic bacteria.

#### 6.2.2.7 Oxazolidinones

The oxazolidinone class is a class of antimicrobial agents with a unique mechanism of action that inhibits protein synthesis. The first agent approved in this class was linezolid which has activity against gram-positive organisms.

#### 6.2.2.8 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.

#### 6.2.2.9 Streptogramins

Streptogramins, which include quinupristin-dalfopristin and linopristin-flopristin, are combinations of two cyclic peptides produced by *Streptomyces* spp. They work synergistically to inhibit protein synthesis, mainly in gram-positive organisms, although they do have limited activity against some gram-negative and anaerobic organisms.

#### 6.2.2.10 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, minocycline, or both. Tigecycline, a glycylcycline, is a derivative of minocycline that has activity against organisms that may be resistant to other tetracyclines.

#### 6.2.2.11 Single-Drug Classes

The following antimicrobial agents (antimicrobial classes) 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), fusidic acid (steroidal), mupirocin (pseudomonic acid), and spectinomycin (aminocyclitols), all of which inhibit protein synthesis; and rifampin (ansamycins) and fidaxomicin (macrocyclics), which are ribonucleic acid (RNA) synthesis inhibitors. 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), approved by the FDA for urinary tract infections only, inhibits enzymes involved in cell wall synthesis.

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## 6.3 Selection Guidelines

To make routine susceptibility testing relevant and practical, the number of agents tested should be limited. M100[1] Tables 1A and 1B 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 collection of random clinical isolates 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, *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 M100[1] Tables 1A and 1B, agents in Group A are considered appropriate for inclusion in a routine, primary testing panel as well as for routine reporting of results for the specified organism groups.

Group B includes antimicrobial agents that may warrant primary testing, but 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 includes 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 epidemiological aid.

Group U includes 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* and ofloxacin).

Group O ("other") includes antimicrobial 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") includes antimicrobial agents that are investigational 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[1] Tables 1A and 1B to report routinely (Group A) and which might be reported only selectively (Group B) in consultation with the infectious disease

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practitioners, the pharmacy, as well as the pharmacy and therapeutics and infection control committees of the health care institution. Selective reporting should 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 tested but not reported routinely should be available on request, or they may be reported for selected specimens. Unexpected resistance, when confirmed, should be reported (eg, resistance to a secondary agent but susceptibility to a primary agent). In addition, each laboratory should develop a protocol to address isolates that are confirmed as resistant to all agents on their routine test panel. This protocol should include options for testing additional agents in-house or sending the isolate to a reference laboratory.

# 7 Reagents for the Disk Diffusion Test

## 7.1 Mueller-Hinton Agar

Of the many media available, the subcommittee considers Mueller-Hinton agar (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.
- A large body of data and experience has 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. If a batch of medium does not support adequate growth of a test organism, zones obtained in a disk diffusion test are usually larger than expected and may exceed the acceptable QC limits. Only MHA formulations that have been tested according to, and that meet the acceptance limits described in, CLSI document M06[16] should be used. Commercially prepared plates may be used or they may be prepared as described in Appendix B.

### 7.1.1 pH

Check the pH of each batch of MHA when the medium is prepared. The agar medium should have a pH between 7.2 and 7.4 at room temperature. The method to assess pH is provided in Appendix B1.1.

### 7.1.2 Moisture

If, just before use, excess surface moisture is present on the plates, place them in an incubator (35°C) or a laminar flow hood at room temperature with lids ajar until excess surface moisture is removed by evaporation (usually 10 to 30 minutes). The surfaces of the plates should be moist, but no droplets of moisture should be apparent on the surface of the medium or on the Petri dish covers when the plates are inoculated.

### 7.1.3 Effects of Thymidine or Thymine

MHA containing excessive amounts of thymidine or thymine can reverse the inhibitory effect of sulfonamides and trimethoprim, thus yielding smaller and less distinct zones, or even no zone at all, which may result in false-resistance reports. Use MHA that is as low in thymidine content as possible. Should problems with QC of sulfonamides and trimethoprim occur, it might be necessary to check the

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MHA. To evaluate a lot of MHA, *Enterococcus faecalis* ATCC®[a] 29212 or, alternatively, *E. faecalis* ATCC® 33186, may be tested with trimethoprim-sulfamethoxazole disks. Satisfactory media provide essentially clear, distinct zones of inhibition ≥ 20 mm. Unsatisfactory media produce no zone of inhibition, growth within the zone, or a zone of < 20 mm.

### 7.1.4 Effects of Variation in Divalent Cations

Variation in divalent cations, principally magnesium and calcium, affects results of aminoglycoside and tetracycline tests with *P. aeruginosa* strains. Excess cation content reduces zone sizes, whereas low cation content may result in unacceptably large zones of inhibition. Variation in calcium levels also affects the results of daptomycin tests. For daptomycin, insufficient calcium content reduces zone sizes, whereas high calcium content may increase zone sizes; therefore, disk diffusion testing is not reliable for testing daptomycin. Excess zinc ions may reduce zone sizes of carbapenems. Performance tests with each lot of MHA must conform to the control limits listed in M100[1] Table 3A.

## 7.2 Testing Strains That Fail to Grow Satisfactorily

Only aerobic or facultative bacteria that grow well on unsupplemented MHA should be tested on that medium. Certain fastidious species, such as *Haemophilus* spp., *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Streptococcus pneumoniae*, and viridans and beta-hemolytic streptococci do not grow sufficiently on unsupplemented MHA. These organisms require supplements or different media to grow, and they should be tested on the media listed below and described in Appendix B using methods described in this document.

- MHA agar with 5% sheep blood
- *Haemophilus* Test Medium (HTM)
- GC agar base + 1% defined growth supplement

Details for these tests are provided in Section 10 and Appendix C.

## 7.3 Antimicrobial Disks

### 7.3.1 Source of Disks and Information About Disks

Disks should be purchased from reliable commercial vendors. The disks should be accompanied at a minimum by a certificate of analysis stating the concentration for the disks, lot number, and the fact that they performed to established parameters against recommended QC organisms.

### 7.3.2 Storage of Antimicrobial Disks

Cartridges containing commercially prepared paper disks specifically for susceptibility testing are generally packaged to ensure appropriate anhydrous conditions. Store disks as follows:

1. Refrigerate the cartridges at 8°C or below or freeze at −14°C or below until needed. Do not store the disks in a self-defrosting freezer. Sealed packages of disks that contain drugs from the β-lactam class should be stored frozen, except for a small working supply, which may be refrigerated for at most one week. Some labile agents (eg, imipenem, cefaclor, and clavulanic acid combinations) may retain greater stability if stored frozen until the day of use.

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2. Remove the sealed packages containing disk cartridges from the refrigerator or freezer one to two hours before use so they may equilibrate to room temperature before opening. This minimizes the amount of condensation that occurs when warm air contacts cold disks.

3. Once a cartridge of disks has been removed from its sealed package, place it in a tightly sealed, desiccated container for storage. If a disk-dispensing apparatus is used, it should be fitted with a tight cover and supplied with an adequate desiccant. Allow the dispenser to warm to room temperature before opening. Avoid excessive moisture by replacing the desiccant when the indicator changes color.

4. When not in use, refrigerate the dispensing apparatus containing the disks.

5. Use only those disks that have not reached the manufacturer's expiration date stated on the label. Discard disks when they reach the expiration date.

# 8 Inoculum Preparation for Disk Diffusion Tests

## 8.1 Turbidity Standard for Inoculum Preparation

To standardize the inoculum density for a susceptibility test, use a $BaSO_4$ turbidity standard equivalent to a 0.5 McFarland standard or its optical equivalent (eg, latex particle suspension). Prepare a $BaSO_4$ 0.5 McFarland standard as described in Appendix B. Alternatively, a photometric device can be used.

## 8.2 Inoculum Preparation

### 8.2.1 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; it is the recommended method for testing the fastidious organisms, *Haemophilus* spp., *N. gonorrhoeae, N. meningitidis*, and streptococci (see Section 10), 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 standard. This results in a suspension containing approximately 1 to $2 \times 10^8$ colony-forming units (CFU)/mL for *Escherichia 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.

### 8.2.2 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.

1. Select at least three to five well-isolated colonies of the same morphological 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 to 5 mL of a suitable broth medium, such as tryptic soy broth.

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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 $2 \times 10^8$ 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 Procedure for Performing the Disk Diffusion Test

## 9.1 Inoculation of Test Plates

1. Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, dip a sterile cotton swab into the adjusted suspension. Rotate the swab several times and press firmly on the inside wall of the tube above the fluid level. This removes excess fluid from the swab.

2. Inoculate the dried surface of an MHA plate by streaking the swab over the entire sterile agar surface. Repeat this procedure by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, swab the rim of the agar.

3. Leave the lid ajar for three to five minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug-impregnated disks.

   **NOTE:** Avoid extremes in inoculum density. Never use undiluted overnight broth cultures or other unstandardized inocula for streaking plates.

## 9.2 Application of Disks to Inoculated Agar Plates

1. Dispense the predetermined battery of antimicrobial disks onto the surface of the inoculated agar plate. Each disk must be pressed down to ensure complete contact with the agar surface. Whether the disks are placed individually or with a dispensing apparatus, they must be distributed evenly so they are no closer than 24 mm from center to center. Ordinarily, no more than 12 disks should be placed on one 150-mm plate, or more than five disks on a 100-mm plate. In all cases, however, it is best to place disks that give predictably small zones (eg, gentamicin, vancomycin) next to those that give larger zones (eg, cephalosporins) in an effort to avoid overlapping zones. It is also important to pay attention to how close the disks are to the edge of the plate, no matter how many disks are dispensed. If disks are placed too close to the edge of the plate, the zones may not be fully round with some drugs. Because some of the drug diffuses almost instantaneously, a disk should not be relocated once it has come into contact with the agar surface. Instead, place a new disk in another location on the agar. If the D-zone test for inducible clindamycin resistance is performed, see Section 12 and M100[1] Tables 2C and 2H-1 for guidance on disk placement.

2. Invert the plates and place in an incubator set to 35 ± 2°C (testing at temperatures above 35°C may not detect methicillin-resistant staphylococci [MRS]) within 15 minutes after the disks are applied. With the exception of *Haemophilus* spp., *N. gonorrhoeae, N. meningitidis,* and streptococci (see Section 10), do not incubate the plates in an increased $CO_2$ atmosphere, because the interpretive standards were developed by using ambient air incubation, and $CO_2$ significantly alters the size of the inhibitory zones of some agents.


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## 9.3 Reading Plates and Interpreting Results

1. After 16 to 18 hours of incubation (see below, Sections 10 and 11, and Appendix C for exceptions), examine each plate. If the plate was satisfactorily streaked, and the inoculum was correct, the resulting zones of inhibition will be uniformly circular and there will be a confluent lawn of growth. If individual colonies are apparent, the inoculum was too light and the test must be repeated. Measure the diameters of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Measure the zones to the nearest whole millimeter, using sliding calipers or a ruler, which is held on the back of the inverted Petri plate. Hold the Petri plate a few inches above a black, nonreflecting background illuminated with reflected light, with the following exceptions:

   - If blood was added to the agar base (as with streptococci), measure the zones from the upper surface of the agar illuminated with reflected light and with the cover removed.

   - If testing oxacillin, cefoxitin, methicillin, or nafcillin, against *Staphylococcus* spp., 24 hours of incubation are required before reporting as susceptible; other agents should be read and reported at 16 to 18 hours. Use transmitted light (plate held up to light) to examine the oxacillin zone for light growth of resistant colonies within apparent zones of inhibition. Any discernable growth within the zone of inhibition is indicative of oxacillin resistance.

   - If testing vancomycin against *S. aureus* or *Enterococcus* spp., 24 hours of incubation are required before reporting as susceptible; other agents should be read and reported at 16 to 18 hours. Disk diffusion testing of vancomycin is not recommended for coagulase-negative staphylococci. For details on methods for detection of reduced susceptibility to vancomycin, see Section 11.1.3.1.

   - If cefoxitin is tested against *Staphylococcus* spp., read the zone diameters with reflected, not transmitted, light.

   - If linezolid is tested against *Staphylococcus* spp., read the zone diameters with transmitted light.

2. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth.

   - However, when discrete colonies grow within a clear zone of inhibition, the test should be repeated with a pure culture or subculture of a single colony from the primary culture plate. If discrete colonies continue to grow within the zone of inhibition, measure the colony-free inner zone.

   - Strains of *Proteus* spp. may swarm into areas of inhibited growth around certain antimicrobial agents. For *Proteus* spp., ignore the thin veil of swarming growth in an otherwise obvious zone of inhibition.

   - When blood-supplemented medium for testing streptococci is used, measure the zone of growth inhibition, not the zone of inhibition of hemolysis.

   - For trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth), and measure the more obvious margin to determine the zone diameter.

3. Interpret the sizes of the zones of inhibition by referring to M100[1] Tables 2A through 2I, and report the organisms as susceptible, intermediate, or resistant to the agents that have been tested (see Section

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14). Some agents may be reported only as susceptible or nonsusceptible, because only susceptible breakpoints are given, as no or very few resistant strains have been identified.

## 10 Fastidious Organisms

Mueller-Hinton medium described previously for the rapidly growing aerobic pathogens is not adequate for susceptibility testing of fastidious organisms. If disk diffusion tests are performed with fastidious organisms, the medium, QC procedures, and interpretive criteria must be modified to fit each organism. Disk diffusion tests for *H. influenzae, N. gonorrhoeae, N. meningitidis, S. pneumoniae,* and beta-hemolytic and viridans group streptococci have been shown to be accurate for selected agents; they are described in Sections 10.1 to 10.4. Other fastidious bacteria may be tested by a dilution or disk diffusion method as described in CLSI document M45.[6] Anaerobic bacteria should not be tested by the disk diffusion test. See CLSI document M11[5] for appropriate anaerobe testing procedures.

### 10.1 *Haemophilus influenzae* and *Haemophilus parainfluenzae*

The medium of choice for disk diffusion testing of *Haemophilus* spp. is HTM.[17] This method has been validated for *H. influenzae* and *H. parainfluenzae* only. (When *Haemophilus* spp. is used below, it applies only to these two species. See CLSI document M45[6] for testing and reporting recommendations for other species of *Haemophilus*.) Instructions for media preparation are provided in Appendix B, or the media may be obtained commercially. Mueller-Hinton chocolate agar is not recommended for routine testing of *Haemophilus* spp.

#### 10.1.1 Test Procedure

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

1. Use the direct colony suspension procedure as described in Section 8.2.1 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 Mueller-Hinton broth (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. This suspension will contain approximately 1 to $4 \times 10^8$ 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. Use the suspension for plate inoculation within 15 minutes after adjusting the turbidity.

2. In general, place no more than nine disks on a 150-mm plate and no more than four disks on a 100-mm plate.

3. Incubate the plates at $35 \pm 2°C$ in an atmosphere of 5% $CO_2$ for 16 to 18 hours before measuring the zones of inhibition (see Section 9.3 [2]).

#### 10.1.2 Zone Diameter Interpretive Criteria

The antimicrobial agents suggested for routine testing of *Haemophilus* spp. are listed in M100[1] Table 1B. Specific zone diameter interpretive criteria used when testing *Haemophilus* spp. are listed in M100[1] Table 2E. Disk diffusion testing of *Haemophilus* spp. with other antimicrobial agents is not recommended.

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## 10.2 *Neisseria gonorrhoeae*

The recommended medium for testing *N. gonorrhoeae* is GC agar to which a 1% defined growth supplement is added after autoclaving.[18] Instructions for preparation are provided in Appendix B, or the agar may be obtained commercially. Cysteine-free growth supplement is not required for disk testing as it is for certain agents with dilution testing (see CLSI document M07).[4] Enriched chocolate agar is not recommended for susceptibility testing of *N. gonorrhoeae.*

### 10.2.1 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* as described in Section 8.2.1. Using colonies taken directly from an overnight chocolate agar culture plate incubated in 5% $CO_2$, prepare a suspension in MHB or saline to a turbidity equivalent to that of a 0.5 McFarland standard. Inoculate plates within 15 minutes after adjusting the turbidity of the inoculum suspension.

2. Place no more than nine antimicrobial disks onto the agar surface of a 150-mm agar plate and no more than four disks onto a 100-mm plate. However, for some agents (eg, fluoroquinolones, cephalosporins) that produce extremely large zones, only two to three disks may be tested per plate.

3. Incubate the plates at 36 ± 1°C (do not exceed 37°C) in an atmosphere of 5% $CO_2$ for 20 to 24 hours before measuring the zones of inhibition (see Section 9.3 [2]).

### 10.2.2 Zone Diameter Interpretive Criteria

The antimicrobial agents suggested for routine testing of *N. gonorrhoeae* are listed in M100[1] Table 1B. Specific zone diameter interpretive criteria used when testing *N. gonorrhoeae* are listed in M100[1] Table 2F. Disk diffusion testing of *N. gonorrhoeae* with other agents is not recommended.

**NOTE:** *N. gonorrhoeae* with 10-μg penicillin disk zone diameters of ≤ 19 mm generally produce β-lactamase.[18] However, β-lactamase tests (see Section 13) are faster than disk diffusion tests and, therefore, are preferred for recognition of this plasmid-mediated penicillin resistance. *N. gonorrhoeae* with plasmid-mediated resistance to tetracycline also has zones of inhibition (30-μg tetracycline disks) of ≤19 mm. Chromosomal mechanisms of resistance to penicillin and tetracycline produce larger zone diameters and can be accurately recognized using the interpretive criteria in M100[1] Table 2F.

## 10.3 *Neisseria meningitidis*

**Recommended precautions:** Perform all antimicrobial susceptibility testing (AST) of *N. meningitidis* in a biological safety cabinet (BSC).[19-21] Manipulating *N. meningitidis* outside a BSC is associated with increased 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 AST) are performed on *N. meningitidis* isolates.

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 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.

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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 (http://www.cdc.gov/vaccines/recs/acip). 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.

Disk diffusion testing of *N. meningitidis* has been validated for detection of possible emerging resistance for some antibiotics but not for penicillins. To date, resistance has mostly been found in 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 disease has not been detected, routine testing of isolates by clinical laboratories is not necessary.

The recommended medium for disk diffusion testing of *N. meningitidis* is MHA supplemented with 5% sheep blood. Instructions for medium preparation are provided in Appendix B, or the medium may be obtained commercially. Enriched chocolate agar is not recommended for susceptibility testing of *N. meningitidis* except as a growth medium for inoculum preparation.

### 10.3.1 Test Procedure

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

1. Use the direct colony suspension procedure when testing *N. meningitidis* as described in Section 8.2.1. Using colonies taken directly from a chocolate agar culture plate incubated for 20 to 22 hours at 35 ± 2°C in an atmosphere of 5% $CO_2$, prepare a suspension in MHB or saline to a turbidity equivalent to that of a 0.5 McFarland standard. Inoculate plates within 15 minutes after adjusting the turbidity of the inoculum suspension.

2. Place no more than five antimicrobial disks onto the agar surface of a 150-mm agar plate and no more than two on a 100-mm agar plate.

3. Incubate the plates at 35 ± 2°C in an atmosphere of 5% $CO_2$ for 20 to 24 hours before measuring the zones of inhibition (see Section 9.3 [2]).

### 10.3.2 Zone Diameter Interpretive Criteria

Specific zone diameter interpretive criteria used when testing *N. meningitidis* are listed in M100[1] Table 2I. Disk diffusion testing of *N. meningitidis* with other agents is not recommended.

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

The recommended medium for testing *S. pneumoniae* and other streptococci is MHA supplemented with 5% sheep blood. Instructions for preparation are provided in Appendix B, or the medium may be obtained commercially.

### 10.4.1 Test Procedure

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

1. Use the direct colony suspension procedure as described in Section 8.2.1 when testing streptococci. Using colonies taken from an overnight (18- to 20-hour) sheep blood agar plate, prepare a suspension in MHB or saline to achieve a turbidity equivalent to a 0.5 McFarland standard. Use the inoculum suspension for plate inoculation within 15 minutes after adjusting the turbidity.

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2. Place no more than nine disks on a 150-mm agar plate and no more than four disks on a 100-mm plate.

3. Incubate the plates at 35 ± 2°C in an atmosphere of 5% $CO_2$ for 20 to 24 hours before measuring the zones of inhibition (see Section 9.3 [2]).

#### 10.4.2 *Streptococcus pneumoniae* Zone Diameter Interpretive Criteria

The antimicrobial agents suggested for routine testing of pneumococci are indicated in M100[1] Table 1B. Specific zone diameter interpretive criteria used when testing *S. pneumoniae* are listed in M100[1] Table 2G.

**NOTE:** For nonmeningitis isolates of *S. pneumoniae,* oxacillin zone sizes of ≥ 20 mm indicate susceptibility to penicillin (oral or parenteral), ampicillin (oral or parenteral), ampicillin-sulbactam, cefaclor, cefdinir, cefditoren, cefpodoxime, cefprozil, ceftizoxime, cefuroxime, imipenem, loracarbef, and meropenem. Because zones of ≤ 19 mm with the oxacillin disk screening test occur with penicillin-resistant, intermediate, and certain susceptible strains, a penicillin, and cefotaxime, ceftriaxone, or meropenem MIC should be determined on isolates of *S. pneumoniae* for which the oxacillin zones are ≤ 19 mm.[22] For isolates with oxacillin zones ≤ 19 mm, do not report as resistant without performing a penicillin MIC.

#### 10.4.3 Other *Streptococcus* spp. Zone Diameter Interpretive Criteria

The antimicrobial agents suggested for routine testing of other streptococci are indicated in M100[1] Table 1B. Specific zone diameter interpretive criteria used when testing other streptococci are listed in M100[1] Tables 2H-1 and 2H-2.

Oxacillin disk testing to determine penicillin susceptibility of streptococci other than *S. pneumoniae* is not recommended. A penicillin or ampicillin disk may be used to predict susceptibility for beta-hemolytic streptococci only. A penicillin MIC should be determined on isolates of viridans group streptococci from normally sterile body sites (eg, CSF, blood, bone). Penicillin and ampicillin disk diffusion tests are not reliable with viridans group streptococci.

Inducible clindamycin resistance can be identified in beta-hemolytic streptococci using the method described in Section 12.

## 11 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.

### 11.1 Staphylococci

#### 11.1.1 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 staphylococci 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 amoxicillin, ampicillin, azlocillin, carbenicillin, mezlocillin, piperacillin, and ticarcillin.

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Some β-lactamase–producing staphylococcal isolates test susceptible to penicillin. Because staphylococcal β-lactamase is readily inducible, there is a risk of this occurring if penicillin were used to treat such strains. For this reason it is recommended that isolates of *Staphylococcus* with penicillin MICs ≤0.12 µg/mL or zone diameters ≥29 mm be tested for β-lactamase production before reporting the isolate as penicillin susceptible. Several tests for β-lactamase production have been described. These include nitrocefin-based tests or evaluating the zone edge of a penicillin disk diffusion test (ie, a fuzzy zone edge indicates no β-lactamase production, whereas a sharp edge indicates β-lactamase production).[23] The penicillin disk diffusion zone-edge test was more sensitive than nitrocefin-based tests for detection of β-lactamase production in *S. aureus.* The penicillin zone-edge test is recommended if only one test is used for β-lactamase detection in *S. aureus.* However, some laboratories may choose to perform a nitrocefin-based test first, and if this test is positive report the results as positive for β-lactamase (or penicillin resistant). If the nitrocefin test is negative, it is recommended that the penicillin disk diffusion zone-edge test be performed before reporting penicillin susceptibility results in cases in which penicillin may be used for therapy for *S. aureus.* For coagulase-negative staphylococci, including *Staphylococcus lugdunensis,* only nitrocefin-based tests are recommended. For the most current recommendations to detect β-lactamases in staphylococcal species, see M100[1] Table 2C.

### 11.1.2 Methicillin/Oxacillin Resistance

#### 11.1.2.1 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 *mec*A 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.

#### 11.1.2.2 Organism Groups

*S. 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, *mec*A-negative strains exhibit oxacillin MICs in the range of 0.25 to 1 µg/mL, whereas *mec*A-positive strains usually exhibit MICs ≥ 4 µg/mL, characteristics more like *S. aureus* than other coagulase-negative staphylococci. Therefore, the presence of *mec*A-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[1] Table 2C. Oxacillin and cefoxitin testing methods for coagulase-negative staphylococci exclude *S. lugdunensis.*

#### 11.1.2.3 Methods for Detection of Oxacillin Resistance

Either oxacillin- or cefoxitin-based methods can be used for detection of *mec*A-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 *mec*A-mediated resistance only; their use is preferred to tests using oxacillin because they are better predictors of the presence of *mec*A than are oxacillin-based methods, including the oxacillin salt-agar screening plate. Because of the rare occurrence of oxacillin resistance mechanisms other than *mec*A in *S. aureus,* some

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*S. aureus* may be encountered that are oxacillin resistant but *mec*A 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[1] Table 2C.

11.1.2.4 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, 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.

- For 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 *mec*A 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 Section 11.1.2.5 for reporting oxacillin when using cefoxitin as a surrogate test).

11.1.2.5 Cefoxitin-Based Methods

- The results of tests using cefoxitin (either broth microdilution or disk diffusion tests using a 30-µg cefoxitin disk) and alternative breakpoints (see M100[1] Table 2C) can be used to predict *mec*A-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 *mec*A-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 disk diffusion testing of *S. lugdunensis,* only the cefoxitin disk test should be used.

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- 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.

11.1.2.6 Molecular Detection Methods

Tests for the *mec*A gene or the protein produced by *mec*A, PBP 2a (also called PBP2′), are the most accurate methods for prediction of resistance to oxacillin.

11.1.2.7 Reporting

- Resistance may be reported any time growth is observed after a minimum of 16 hours of 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 *mec*A, or that produce PBP 2a, the *mec*A gene product, as oxacillin resistant. Report isolates that do not carry *mec*A or do not produce PBP 2a as oxacillin susceptible.

- Report oxacillin-resistant staphylococci as resistant to all other penicillins, carbapenems, cephems (with the exception of the cephalosporins with anti-MRSA activity), and β-lactam/β-lactamase inhibitors, regardless of *in vitro* test results for 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.

### 11.1.3 Vancomycin Resistance in *S. aureus*

In 2006 (M100-S16[24]), the interpretive criteria for vancomycin and *S. aureus* were lowered to ≤2 µg/mL for susceptible, 4 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 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 to 16 µg/mL) was reported from Japan in 1997,[25] followed by reports from the United States and France.[26] 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* strains appear to have developed from MRSA.

Since 2002, *S. aureus* strains for which the vancomycin MICs ranged from 32 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.[27,28] These strains are reliably detected by the broth microdilution reference method, the disk diffusion method, and the vancomycin agar screen test (see Section 11.1.3.1) when the tests are incubated for a full 24 hours at 35 ± 2°C.

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11.1.3.1 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 to 16 µg/mL, MIC testing must be performed and the tests incubated for a full 24 hours at 35 ± 2°C. 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.

**Ability of Various Methods to Detect Levels of Vancomycin Susceptibility in *S. aureus***

| Vancomycin MIC (µg/mL) | MIC Method | Disk Diffusion Method* | Vancomycin Agar Screen |
|---|---|---|---|
| ≤2 (S) | Yes | No | Yes |
| 4 (I) | Yes | No | Variable |
| 8 (I) | Yes | No | Yes |
| 16 (R) | Yes | No | Yes |
| ≥32 (R) | Yes | Yes | Yes |

* Strains of *S. aureus* for which the vancomycin zone diameter is ≥7 mm may have MICs from ≤2 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.

11.1.3.2 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 8.2.1).

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 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, because it may give false-positive results.)

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- *E. faecalis* ATCC® 51299 (vancomycin resistant) – positive control.

7. Do not reuse plates after incubation.

Many *S. aureus* isolates with vancomycin MICs of 4 µg/mL do not grow on this vancomycin agar screen media (see Section 11.1.3.1). Also, there are insufficient data to recommend using this agar screen test for coagulase-negative staphylococci.

#### 11.1.3.3 Heteroresistant Vancomycin-Intermediate *Staphylococcus aureus*[29]

When first described in 1997, heteroresistant vancomycin-intermediate *Staphylococcus aureus* (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 to 16 µg/mL, ie, in the intermediate range. Because a standard broth microdilution test uses an inoculum of $5 \times 10^5$ 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 to 4 µg/mL, and the resistant breakpoint from ≥ 32 to ≥ 16 µg/mL to make the breakpoints more predictive of clinical outcome. Thus, the vancomycin-susceptible breakpoint for *S. aureus* is now ≤ 2 µg/mL, the intermediate range is 4 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 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[30,31]) has become the *de facto* best available method for investigating the clinical relevance of hVISA strains in several large surveillance studies. However, this technique 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.

#### 11.1.3.4 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. See M100[1] Table 2C for most recent recommendations for testing and reporting.

### 11.1.4 Inducible Clindamycin Resistance

Inducible clindamycin resistance can be identified using the method described in Section 12, and in Section 13 of CLSI document M07.[4]

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### 11.1.5 Linezolid Resistance

When testing linezolid by disk diffusion, zones should be examined using transmitted light after incubation for 16 to 18 hours at 35 ± 2°C.

### 11.1.6 Mupirocin Resistance

Rates of high-level mupirocin resistance may increase (ie, MICs ≥ 512 µg/mL) in *S. aureus* and are associated with the presence of the plasmid-mediated *mupA* gene.[32-34] High-level mupirocin resistance can be detected using either routine disk diffusion or broth microdilution tests.[35] 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.

## 11.2 Enterococci

### 11.2.1 Penicillin/Ampicillin Resistance

Enterococci may be resistant to penicillin and ampicillin because of production of low-affinity, 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 13.2). 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 may not be susceptible to the synergistic effect.[36,37] Physicians' requests to determine the actual MIC of penicillin or ampicillin for blood and CSF isolates of enterococci should be considered.

### 11.2.2 Vancomycin Resistance

Accurate detection of vancomycin-resistant enterococci (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 in Section 11.2.3 and in M100[1] Table 2D Supplemental Table 1.

### 11.2.3 Vancomycin Agar Screen

The vancomycin agar screening-plate procedure can be used in addition to the dilution methods described in Section 11.2.2 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 of vancomycin/mL.[38]

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

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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 to 15 mm in diameter.

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

3. Incubate the plate at 35 ± 2°C in ambient air for a full 24 hours and examine carefully, using transmitted light, for evidence of growth, including small colonies (> 1 colony) or a film of growth, indicating vancomycin resistance (also see M100[1] Table 2D Supplemental Table 1).

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.

### 11.2.4 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.[36] 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 M100[1] Table 2D Supplemental Table 1). QC of these tests is also explained in M100[1] Table 2D Supplemental Table 1. Other aminoglycosides need not be tested, because their activities against enterococci are not superior to gentamicin or streptomycin.

## 11.3 Gram-Negative Bacilli

### 11.3.1 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. β-lactamases may be named after the primary substrates that they hydrolyze, the biochemical properties of the β-lactamases, strains of bacteria from which the β-lactamase was detected, a patient from whom a β-lactamase–producing strain was isolated, etc.[39] For example, TEM is an abbreviation for Temoneira, the first patient from whom a TEM β-lactamase–producing strain was reported. β-lactamases may be classified as molecular Class A, B, C, or D enzymes.[40]

| Class | Active Site | Examples |
|---|---|---|
| A | Inhibitor-susceptible (rare exceptions) | TEM-1, SHV-1, KPCs, OXY, and most ESBLs (including CTX-M) |
| B | Metallo-β-lactamases | Metalloenzymes; VIM, IMP, SPM, NDM |
| C | Inhibitor-resistant β-lactamases | AmpC |
| D | Oxacillin-active β-lactamases that may be inhibitor susceptible | OXA (including rare ESBL phenotypes) |

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β-lactamase enzymes in all four classes inactivate β-lactam antimicrobial agents at different rates. The genes encoding β-lactamases may be located on chromosomes and expressed with or without induction or carried on plasmids in single or multiple copies. An isolate may produce β-lactamases and possess other resistance mechanisms such as porin mutations that restrict antimicrobial access to their active binding sites in the bacterial cell. The variety of β-lactam resistance mechanisms encountered in gram-negative bacteria gives rise to a continuum of antimicrobial activities expressed as a range of MIC values. One would expect the interpretive breakpoint to be the MIC or zone diameter value that differentiates β-lactamase/other resistance mechanism–negative strains (susceptible) from β-lactamase/other resistance mechanism–positive stains (resistant). However, weak β-lactamase activity or low-level β-lactamase expression may not necessarily mean that the isolate will be refractory to β-lactam therapy. In practice, some isolates that are interpreted as susceptible will produce β-lactamases that have clinically inconsequential enzyme activity. These may be ESBL, AmpC, or carbapenemase-type enzymes as described in Sections 11.3.2, 11.3.3, and 11.3.4.

Identification of a specific β-lactamase resistance mechanism (eg, ESBL, *Klebsiella pneumoniae* carbapenemase [KPC], NDM) is not required or necessary for the determination of a susceptible or resistant interpretation. However, the identification of a specific enzyme may be useful for infection control procedures or epidemiological investigations. The M100[1] Supplemental Tables with Table 2A describe tests that can be used to screen for and confirm the presence of ESBLs in *E. coli, K. pneumoniae, Klebsiella oxytoca,* and *Proteus mirabilis,* and carbapenemase production in *Enterobacteriaceae.*

### 11.3.2 Extended-Spectrum β-Lactamases

ESBLs are inhibitor-susceptible enzymes in Classes A and D 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. ESBLs may confer resistance to penicillins, cephalosporins, and aztreonam in clinical isolates of *K. pneumoniae, K. oxytoca, E. coli, P. mirabilis,*[41] and other genera of the family *Enterobacteriaceae.*[40] When using revised CLSI cephalosporin and aztreonam breakpoints first published in M100-S20,[42] most ESBL-negative strains will test susceptible; however, some strains that test susceptible may contain ESBL genes that code for production of low amounts of enzyme or enzyme that has poor hydrolytic activity. These strains are categorized correctly as susceptible.

A similar native enzyme, OXY (formerly KOXY or K1), in *K. oxytoca* acts as an extended-spectrum penicillinase, inactivating amino- and carboxypenicillins. When OXY enzymes are overproduced as a result of promoter mutations, resistance to ceftriaxone and aztreonam (but not ceftazidime), as well as resistance to all combinations of β-lactams and β-lactamase inhibitors, results. Although strains producing OXY enzymes may result in a positive ESBL confirmatory test, OXY enzymes are generally not considered ESBLs. MIC and zone diameter values correctly predict susceptible and resistant interpretations.

### 11.3.3 AmpC Enzymes

The AmpC β-lactamases are chromosomal or plasmid-encoded enzymes.[43] Isolates that produce AmpC enzymes have a similar antimicrobial susceptibility profile to those that produce ESBLs in that they show reduced susceptibility to penicillins, cephalosporins, and aztreonam. However, in contrast to ESBLs, AmpC β-lactamases also inactivate cephamycins, ie, bacteria expressing AmpC enzyme test as resistant to cefoxitin and cefotetan. In addition, AmpC-producing strains are resistant to the current β-lactamase inhibitor combination agents, and strains producing AmpC enzyme may test resistant to carbapenems if accompanied by a porin mutation or in combination with overexpression of specific efflux pumps.

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Chromosomal AmpC β-lactamases are found in *Enterobacter*, *Citrobacter*, *Serratia*, and some other gram-negative species, and 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 III and IV) do not induce AmpC enzymes but can be hydrolyzed by them. Use of cephalosporins also may select for stably derepressed chromosomal mutants, which can emerge during therapy.[44]

AmpC enzymes can be carried on plasmids that are transmissible among bacteria. Although plasmid-mediated AmpC enzymes evolved from native chromosomal enzymes among a diverse group of bacteria, they are found primarily in clinical isolates of *K. pneumoniae* and *E. coli.*

There are no CLSI-validated phenotypic tests to confirm the presence of plasmid-encoded AmpC β-lactamases in clinical isolates. Strains carrying both ESBLs and plasmid-encoded AmpC β-lactamases are common in some geographical regions. The current susceptibility breakpoints for drugs affected by these combinations of enzymes, first published in M100-S20,[42] are the best approach for providing guidance for treatment of these strains.

### 11.3.4 Carbapenemases (Carbapenem-Resistant *Enterobacteriaceae*)

Carbapenemase activity in clinical isolates of *Enterobacteriaceae* occurs as a result of β-lactamase enzymes in Classes A, B, and D. KPC-type enzymes within Class A, NDM-type enzymes within Class B, and OXA-type enzymes within Class D represent major families of clinical importance (see the table below). The presence of KPC-type enzymes can be confirmed using the modified Hodge test as described in M100[1] Supplemental Tables with Table 2A. NDM-type and other metallo-β-lactamase enzymes require zinc for activity and are inhibited by substances such as ethylenediaminetetraacetic acid (EDTA), which binds zinc. *Stenotrophomonas maltophilia, Bacillus anthracis,* and some strains of *Bacteroides fragilis* produce a chromosomal metallo-β-lactamase. Other metalloenzymes may be carried on plasmids and can occur in *Acinetobacter* spp., *P. aeruginosa, Serratia marcescens, K. pneumoniae,* and, increasingly, in other *Enterobacteriaceae.* There are no CLSI-validated phenotypic tests to confirm the presence of metallo-β-lactamases in clinical isolates. Current susceptibility breakpoints for drugs affected by these carbapenemases, first published in M100-S20-U,[45] are the recommended approach for providing guidance for treatment of infection by *Enterobacteriaceae* containing KPC- and NDM-type enzymes.

**β-Lactamases With Carbapenemase Activity**

| β-Lactamase Class* | Found In | Examples |
|---|---|---|
| A | *K. pneumoniae* and other *Enterobacteriaceae* | KPC, SME |
| B | *Enterobacteriaceae*<br>*P. aeruginosa*<br>*Acinetobacter baumannii* | Metallo-β-lactamases inhibited by EDTA<br>(IMP, VIM, NDM) |
| D | *A. baumannii*<br>*Enterobacteriaceae* | OXA |

* Carbapenemases have not yet been found in Class C.

## 11.4 *Streptococcus pneumoniae*

### 11.4.1 Penicillin and Third-Generation Cephalosporin Resistance

Penicillin, and cefotaxime, 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[1] Table 2G for reporting of penicillins and third-generation cephalosporins, because there are specific interpretive criteria that must be used

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depending on the site of infection and the penicillin formulation used for therapy. In M100[1] Table 2G, breakpoints are listed for intravenous penicillin therapy for meningitis and nonmeningitis infections. Separate breakpoints are included 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.

## 12 Inducible Clindamycin Resistance

Macrolide-resistant isolates of *S. aureus,* coagulase-negative *Staphylococcus* spp., and β-hemolytic streptococci may express constitutive or inducible resistance to clindamycin (methylation of the 23S ribosomal RNA 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 an *mef* gene in streptococci). Infections caused by staphylococci with inducible clindamycin resistance may fail to respond to clindamycin therapy.[46] However, the clinical significance of inducible resistance in streptococci is unclear. Inducible clindamycin resistance can be detected for all staphylococci and β-hemolytic streptococci using a disk diffusion test with clindamycin and erythromycin disks placed in proximity[13,14] or as a single well test using broth microdilution.

Using disk diffusion, the test is done by placing a 2-µg clindamycin disk either 15 to 26 mm away (for staphylococci) or 12 mm away (for streptococci) from the edge of a 15-µg erythromycin disk on a standard blood agar plate used for the inoculum purity check or by using the standard disk diffusion procedure with MHA. 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 in an erythromycin-resistant isolate should be reported as tested (ie, susceptible or intermediate to clindamycin).

For staphylococci and β-hemolytic streptococci, inducible clindamycin resistance can also be detected with the broth microdilution test using the combination of erythromycin and clindamycin. For staphylococci, erythromycin 4 µg/mL and clindamycin 0.5 µg/mL are used together in a single well of a broth microdilution panel; for streptococci, the combination of erythromycin 1 µg/mL and clindamycin 0.5 µg/mL is used. The broth microdilution test applies only to isolates that are erythromycin resistant and clindamycin susceptible or intermediate; 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 show flattening of the clindamycin zone adjacent to the erythromycin disk (referred to as a D-zone) or grow in the single combination well have inducible clindamycin resistance. Recommendations for QC and the most recent updates for testing are provided in M100[1] Tables 2C, 2H-1, 3A, and 3B.

## 13 β-Lactamase Tests

### 13.1 Purpose

A rapid β-lactamase test may yield clinically relevant results earlier than a disk diffusion test with *Haemophilus* spp., *Moraxella catarrhalis,* and *N. gonorrhoeae;* a β-lactamase test is the only reliable test for detecting β-lactamase–producing *Enterococcus* spp.

A positive β-lactamase test result predicts the following:

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- Resistance to penicillin, ampicillin, and amoxicillin among *Haemophilus* spp., *M. catarrhalis,* and *N. gonorrhoeae*

- Resistance to penicillin, and amino-, carboxy-, and ureidopenicillins among staphylococci and enterococci

A negative β-lactamase test result does not rule out β-lactam 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.

### 13.2 Selecting a β-Lactamase Test

Nitrocefin-based tests are the preferred method for testing enterococci, *Haemophilus* spp., *M. catarrhalis,* and *N. gonorrhoeae.*[47] 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.*[48] Accurate detection of β-lactamase in staphylococci may require alternative methods, such as induction of the enzyme or evaluation of the penicillin disk diffusion zone-edge for *S. aureus* (see Section 11.1.1 and M100[1] Table 2C). 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 for commercial tests).

## 14 Interpretation of Disk Diffusion Test Results

### 14.1 Zone Diameter Interpretive Standards

Zone diameter interpretive criteria to categorize the levels of susceptibility of organisms to various antimicrobial agents are provided in M100[1] Tables 2A through 2J. For most agents, these criteria are developed by first comparing zone diameters to MICs of a large number of isolates, including those with known mechanisms of resistance relevant to the particular class of drug. Second, the MICs and correlated zone sizes 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 microbiological eradication efficacy in the treatment of specific pathogens, as outlined in CLSI document M23.[3]

### 14.2 Interpretive Categories

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

## 15 Quality Control and Quality Assurance Procedures

### 15.1 Purpose

In AST, 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 monitor the following:

- Precision (repeatability) and accuracy of susceptibility test procedures
- Performance of reagents used in the tests


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- 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. QA includes, but is not limited to, monitoring, evaluating, taking corrective actions (if necessary), recordkeeping, calibration and maintenance of equipment, proficiency testing, training, and QC.

## 15.2 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 only to 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 management system standards)
  - Integrity of product
  - 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
  - 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 are most likely to cause user performance problems and to determine that the test is performing correctly when used according to established protocols.

## 15.3 Selection of 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, 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.[3] Users of commercial systems should follow the QC recommendations in their instructions for use.

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QC strains and their characteristics are described in Appendix D. Some of these are listed as "QC strains" and others as "supplemental QC strains." These can be defined as follows:

QC strains are tested regularly (eg, daily, weekly) to ensure the test system performs as expected and produces results that fall within specified limits listed in M100.[1] The QC strains recommended in this document should be included if a laboratory performs CLSI reference disk diffusion testing as described herein. For commercial test systems, manufacturers' 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, *H. 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 *H. influenzae* and *H. parainfluenzae.* Supplemental QC strains may possess susceptibility or resistance characteristics specific for one or more special tests listed in CLSI documents M02 and M07.[4] 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[1] Tables 3A and 3B.

## 15.4 Storing and Testing Quality Control Strains

- Test the QC strains by standard disk diffusion 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 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 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 (eg, 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 (eg, subculture each week for no more than three successive weeks). For best results, some strains may require preparation of new subcultures more frequently (eg, every two weeks).

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- If an unexplained result suggests a change in the organism's inherent susceptibility, prepare a new primary subculture or working culture, or obtain a fresh stock culture of the QC strain. See Section 15.8 for additional guidance.

## 15.5 Batch or Lot Quality Control

1. Test each new batch or lot of agar plates or disks with the appropriate QC strains to determine if zone sizes obtained with the batch or lot fall within the expected range (see M100[1] Tables 3A and 3B); if they do not, the batch or lot must be rejected.

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

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

## 15.6 Zone Diameter Quality Control Limits

Acceptable zone diameter QC limits for a single QC test (single-drug/single-organism combination) are listed in M100[1] Tables 3A and 3B.

## 15.7 Frequency of Quality Control Testing (also refer to Appendix A and M100[1] Table 3C)

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 15.7.2.1), test the QC strains weekly. The weekly QC testing option outlined in Section 15.7.2 is not applicable when disk diffusion tests are performed less than once a week. QC testing should be performed each test day for disk diffusion tests performed less than once a week.

### 15.7.1 Daily Testing

Performance is satisfactory for daily QC testing when no more than three out of 30 results obtained on consecutive test days for each antimicrobial agent/organism combination are outside the acceptable limit stated in M100[1] Tables 3A and 3B. Corrective action by the laboratory is required when this frequency is exceeded (see Section 15.8).

### 15.7.2 Weekly Testing

15.7.2.1 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 one out of 20 or three out of 30 zone diameters for each antimicrobial agent/organism combination may be outside the acceptable zone diameter limits stated in M100[1] Tables 3A and 3B.

15.7.2.2 Implementing Weekly Quality Control Testing

- Weekly QC testing may be performed once satisfactory performance has been documented (see Section 15.7.2.1).

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- Perform QC testing once per week and whenever any reagent component of the test (eg, a new lot of agar or a new lot of disks from the same or a different manufacturer) is changed.

- If any of the weekly QC results are out of the acceptable range, corrective action is required (see Section 15.8).

- Refer to M100[1] Table 3C for guidance on QC frequency with new materials or test modifications.

## 15.8 Corrective Action

### 15.8.1 Out-of-Control Result Due to Identifiable Error

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[1] Table 3D 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
  - Changes in the organism (eg, mutation, loss of plasmid)

- Testing supplies
  - Improper storage or shipping conditions
  - Contamination
  - Use of a defective agar plate (eg, too thick or too thin)
  - Use of damaged (eg, cracked) plates
  - 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 disk, ancillary supplies
  - Improper disk placement (eg, inadequate contact with the agar)
  - Incorrect reading or interpretation of test results
  - Transcription error

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


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### 15.8.2 Out-of-Control Result With No Error Identified

15.8.2.1 Immediate Corrective Action

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

- 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 five consecutive test days. Document all results.

  – If all five zone diameter measurements for the antimicrobial agent/organism combination are within the acceptable ranges, as defined in M100[1] Tables 3A and 3B, no additional corrective action is necessary.

  – If any of the five zone diameter measurements are still outside the acceptable range, additional corrective action is required (see Section 15.8.2.2 and M100[1] Tables 3A and 3B).

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

15.8.2.2 Additional Corrective Action

When immediate corrective action does not resolve the problem, the problem is likely due to a system error rather than a random error. Additional investigation and corrective action is required. Refer to Section 15.8.1 and M100[1] Table 3D, 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 is also 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 alternative test method.

If a problem is identified and 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 test days is required in order to return to weekly QC testing (see Section 15.7.2.1).

## 15.9 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 zone size, slightly or significantly decreased zone size).

- Is the patient result close to the interpretive breakpoint?

- Results with other QC organisms.

- Results with other antimicrobial agents.

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- Is the QC strain/antimicrobial agent an indicator for a procedural or storage issue (eg, inoculum dependent, heat labile)? Refer to M100[1] Table 3D, 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 alternative test method or a reference laboratory until the problem is resolved.

## 15.10 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 before 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*).

- 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.[1]

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 (eg, Is there a similar trend or observation with recent QC testing?)

- Problems with the testing supplies, process, or equipment (see Section 15.8.1 and M100[1] Table 3D, 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 M100[1] Appendix A. 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.

## 15.11 Other Control Procedures

### 15.11.1 Inoculum Control

Periodically ensure that the 0.5 McFarland standard remains under control (see Appendix B2.1).

### 15.11.2 End-point Interpretation Control

Monitor end-point interpretation periodically to minimize variation in the interpretation of zone sizes among observers. All laboratory personnel who perform these tests should independently read a selected

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set of tests. Record the results and compare to the results obtained by an experienced reader; or, when using QC strains, compare to the expected results from M100[1] Tables 3A and 3B.

# 16 Limitations of Disk Diffusion Methods

## 16.1 Application to Various Organism Groups

The disk diffusion methods described in this document are standardized for testing rapidly growing pathogens, which include *Staphylococcus* spp., *Enterococcus* spp., the *Enterobacteriaceae, P. aeruginosa, Acinetobacter* spp., *Burkholderia cepacia,* and *S. maltophilia,* and they have been modified for testing fastidious organisms such as *H. influenzae* and *H. parainfluenzae* (M100[1] Table 2E), *N. gonorrhoeae* (M100[1] Table 2F), *N. meningitidis* (M100[1] Table 2I), and streptococci (M100[1] Tables 2G, 2H-1, and 2H-2). For organisms excluded from M100[1] Tables 2A through 2I and not covered in other CLSI guidelines or standards, such as CLSI document M45,[6] studies are not yet adequate to develop reproducible, definitive standards to interpret results. These organisms may require different media, require different atmospheres of incubation, or show marked strain-to-strain variation in growth rate. For these microorganisms, consultation with an infectious disease specialist is recommended for guidance in determining the need for susceptibility testing and in the interpretation of results. Published reports in the medical literature and current consensus recommendations for therapy of uncommon microorganisms may obviate the need for testing of such organisms. If testing is necessary, a dilution method usually is the most appropriate testing method, and this may require submitting the organism to a reference laboratory.

## 16.2 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, cephamycins, and aminoglycosides against *Salmonella* and *Shigella* spp.

- Penicillins, β-lactam/β-lactamase inhibitor combinations, cephems (except for cephalosporins with anti-MRSA activity), and carbapenems against oxacillin-resistant *Staphylococcus* spp.

- Aminoglycosides (except high concentrations), cephalosporins, clindamycin, and trimethoprim-sulfamethoxazole against *Enterococcus* spp.

## 16.3 Development of Resistance and Testing of Repeat Isolates

Isolates that are initially susceptible may become intermediate or resistant after initiation of therapy. Therefore, subsequent isolates of the same species from a similar body site should be tested in order to detect resistance that may have developed. This can occur within as little as three to four days and has been noted most frequently in *Enterobacter, Citrobacter,* and *Serratia* spp. with third-generation cephalosporins; in *P. aeruginosa* with all antimicrobial agents; and in staphylococci with quinolones. For *S. aureus,* vancomycin-susceptible isolates may become vancomycin intermediate during the course of prolonged therapy.

In certain circumstances, testing of subsequent isolates to detect resistance that may have developed might be warranted earlier than within three to four days. The decision to do so requires knowledge of the specific situation and the severity of the patient's condition (eg, an isolate of *Enterobacter cloacae* from a

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blood culture on a premature infant). Laboratory guidelines on when to perform susceptibility testing on repeat isolates should be determined after consultation with the medical staff.

## 17 Screening Tests

Screening tests, as described in this document and in M100,[1] characterize an isolate as susceptible or resistant to one or more antimicrobial agents based on a specific resistance mechanism or phenotype. Some screening tests have sufficient sensitivity and specificity such that results of the screen can be reported without additional testing. Others require further testing to confirm the presumptive results obtained with the screen test. The details for each screening test, including test specifications, limitations, and additional tests needed for confirmation, are provided in M100[1] Instructions for Use of Tables 1 and 2, Section VII and in the M100[1] Table 2 locations specified there.


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## References


[1] CLSI. *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement*. CLSI document M100-S22. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.

[2] CLSI. *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement*. CLSI document M100-S21. Wayne, PA: Clinical and Laboratory Standards Institute; 2011.

[3] CLSI. *Development of* In Vitro *Susceptibility Testing Criteria and Quality Control Parameters; Approved Guideline—Third Edition.* CLSI document M23-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.

[4] CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Ninth Edition.* CLSI document M07-A9. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.

[5] CLSI. *Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard—Seventh Edition.* CLSI document M11-A7. Wayne, PA: Clinical and Laboratory Standards Institute; 2007.

[6] CLSI. *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Approved Guideline—Second Edition.* CLSI document M45-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2010.

[7] Ericsson HM, Sherris JC. Antibiotic sensitivity testing. Report of an international collaborative study. *Acta Pathol Microbiol Scand B Microbiol Immunol.* 1971;217(Suppl 217):1-90.

[8] Rules and Regulations, Antibiotic Susceptibility Discs. *Federal Register.* 1972;37:20525-20529.

[9] Rules and Regulations, Antibiotic Susceptibility Discs: Correction. *Federal Register.* 1973;38:2756.

[10] Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol.* 1966;45(4):493-496.

[11] Siegel JD, Rhinehart E, Jackson M, Chiarello L, and the Healthcare Infection Control Practices Advisory Committee. 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings. http://www.cdc.gov/hicpac/pdf/isolation/isolation2007.pdf. Accessed November 3, 2011.

[12] CLSI. *Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Third Edition*. CLSI document M29-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.

[13] Fiebelkorn KR, Crawford SA, McElmeel ML, Jorgensen JH. Practical disk diffusion method for detection of inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci. *J Clin Microbiol*. 2003;41(10):4740-4744.

[14] Jorgensen JH, Crawford SA, McElmeel ML, Fiebelkorn KR. Detection of inducible clindamycin resistance of staphylococci in conjunction with performance of automated broth susceptibility testing. *J Clin Microbiol*. 2004;42(4):1800-1802.

[15] ISO. *Quality management systems – Fundamentals and vocabulary.* ISO 9000. Geneva, Switzerland: International Organization for Standardization; 2005.

[16] CLSI. *Protocols for Evaluating Dehydrated Mueller-Hinton Agar; Approved Standard—Second Edition*. CLSI document M06-A2; Wayne, PA: Clinical and Laboratory Standards Institute; 2006.

[17] Jorgensen JH, Redding JS, Maher LA, Howell AW. Improved medium for antimicrobial susceptibility testing of *Haemophilus influenzae*. *J Clin Microbiol.* 1987;25(11):2105-2113.

[18] Jones RN, Gavan TL, Thornsberry C, et al. Standardization of disk diffusion and agar dilution susceptibility tests for *Neisseria gonorrhoeae:* Interpretive criteria and quality control guidelines for ceftriaxone, penicillin, spectinomycin, and tetracycline. *J Clin Microbiol.* 1989;27(12):2758-2766.

[19] Biosafety in Microbiological and Biomedical Laboratories. 5th ed. US Government Printing Office, Washington, DC: 2007. http://www.cdc.gov/biosafety/publications/bmbl5/index.htm. Accessed November 3, 2011.

[20] World Health Organization. Laboratory Biosafety Manual. 3rd ed. http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/. Accessed November 3, 2011.

[21] Fleming DO, Hunt DL, eds. *Biological Safety: Principles and Practices.* 4th ed. Washington, DC: ASM Press; 2006.

[22] Jorgensen JH, Swenson JM, Tenover FC, Ferraro MJ, Hindler JA, Murray PR. Development of interpretive criteria and quality control limits for broth microdilution and disk diffusion antimicrobial susceptibility testing of *Streptococcus pneumoniae. J Clin Microbiol.* 1994;32(10):2448-2459.

[23] Gill VJ, Manning CB, Ingalls CM. Correlation of penicillin minimum inhibitory concentrations and penicillin zone edge appearance with staphylococcal beta-lactamase production. *J Clin Microbiol.* 1981;14(4):437-440.


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[24] CLSI. *Performance Standards for Antimicrobial Susceptibility Testing; Sixteenth Informational Supplement*. CLSI document M100-S16. Wayne, PA: Clinical and Laboratory Standards Institute; 2006.

[25] Hiramatsu K, Hanaki H, Ino T, Yabuta K, Oguri T, Tenover FC. Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. *J Antimicrob Chemother*. 1997;40(1):135-136.

[26] Fridkin SK. Vancomycin-intermediate and -resistant *Staphylococcus aureus*: what the infectious disease specialist needs to know. *Clin Infect Dis*. 2001;32(1):108-115.

[27] Centers for Disease Control and Prevention. *Staphylococcus aureus* resistant to vancomycin – United States, 2002. *MMWR Morb Mortal Wkly Rep*. 2002;51(26):565-567.

[28] Centers for Disease Control and Prevention. Public health dispatch: vancomycin-resistant *Staphylococcus aureus* – Pennsylvania, 2002. *MMWR Morb Mortal Wkly Rep*. 2002;51:902.

[29] Tenover FC, Moellering RC Jr. The rationale for revising the Clinical and Laboratory Standards Institute vancomycin minimal inhibitory concentration interpretive criteria for Staphylococcus aureus. *Clin Infect Dis.* 2007;44(9):1208-1215.

[30] Hiramatsu K, Aritaka H, Hanaki H, et al. Dissemination in Japanese hospitals of strains of *Staphylococcus aureus* heterogeneously resistant to vancomycin. *Lancet.* 1997;350(9092):1670-1673.

[31] Wootton M, Howe RA, Hillman R, Walsh TR, Bennett PM, MacGowan AP. A modified population analysis profile (PAP) method to detect hetero-resistance to vancomycin in *Staphylococcus aureus* in a UK hospital. *J Antimicrob Chemother.* 2001;47(4):399-403.

[32] Simor AE. Randomized controlled trial of chlorhexidine gluconate for washing, intranasal mupirocin, and rifampin and doxycycline vs no treatment for the eradication of methicillin-resistant *Staphylococcus aureus* colonization. *Clin Infec Dis.* 2007;44(2):178-185.

[33] Simor AE, Stuart TL, Louie L, et al. Mupirocin-resistant, methicillin-resistant *Staphylococcus aureus* strains in Canadian hospitals. *Antimicrob Agents Chemother.* 2007;51(11):3880-3886.

[34] Jones JC, Rogers TJ, Brookmeyer P, et al. Mupirocin resistance in patients colonized with methicillin-resistant *Staphylococcus aureus* in a surgical intensive care unit. *Clin Infect Dis.* 2007;45(5):541-547.

[35] Swenson JM, Wong B, Simor AE, et al. Multicenter study to determine disk diffusion and broth microdilution criteria for prediction of high- and low-level mupirocin resistance in *Staphylococcus aureus. J Clin Microbiol*. 2010;48(7):2469-2475.

[36] Torres C, Tenorio C, Lantero M, Gastãnares MJ, Baquero F. High-level penicillin resistance and penicillin-gentamicin synergy in *Enterococcus faecium. Antimicrob Agents Chemother.* 1993;37(11):2427-2431.

[37] Murray BE. Vancomycin-resistant enterococci. *Am J Med.* 1997;102(3):284-293.

[38] Swenson JM, Clark NC, Ferraro MJ, et al. Development of a standardized screening method for detection of vancomycin-resistant enterococci. *J Clin Microbiol.* 1994;32(7):1700-1704.

[39] Jacoby GA. Beta-lactamase nomenclature. *Antimicrob Agents Chemother*. 2006;50(4):1123-1129.

[40] Jacoby GA, Munoz-Price LS. The new beta-lactamases. *N Engl J Med.* 2005;352(4):380-391.

[41] Bonnet R, De Champs C, Sirot D, Chanal C, Labia R, Sirot J. Diversity of TEM mutants in *Proteus mirabilis*. *Antimicrob Agents Chemother.* 1999;43(11):2671-2677.

[42] CLSI. *Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement*. CLSI document M100-S20; Wayne, PA: Clinical and Laboratory Standards Institute; 2010.

[43] Jacoby GA. AmpC beta-lactamases. *Clin Microbiol Rev.* 2009;22(1):161-182.

[44] Chow JW, Fine MJ, Shlaes DM, et al. Enterobacter bacteremia: clinical features and emergence of antibiotic resistance during therapy. *Ann Intern Med*. 1991;115(8):585-590.

[45] CLSI. *Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement (June 2010 Update)*. CLSI document M100-S20-U; Wayne, PA: Clinical and Laboratory Standards Institute; 2010.

[46] Lewis JS 2nd, Jorgensen JH. Inducible clindamycin resistance in staphylococci: Should clinicians and microbiologists be concerned? *Clin Infect Dis.* 2005;40(2):280-285.

[47] Swenson JM, Patel JB, Jorgensen JH. Special phenotypic tests for detecting antibacterial resistance. In: Versalovic J., Carroll KC, Funke G, et al., eds. *Manual of Clinical Microbiology*. 10th ed. Washington, DC: American Society for Microbiology; 2011:1155-1179.

[48] Doern GV, Tubert TA. Detection of β-lactamase activity among clinical isolates of *Branhamella catarrhalis* with six different β-lactamase assays. *J Clin Microbiol.* 1987;25(8):1380-1383.

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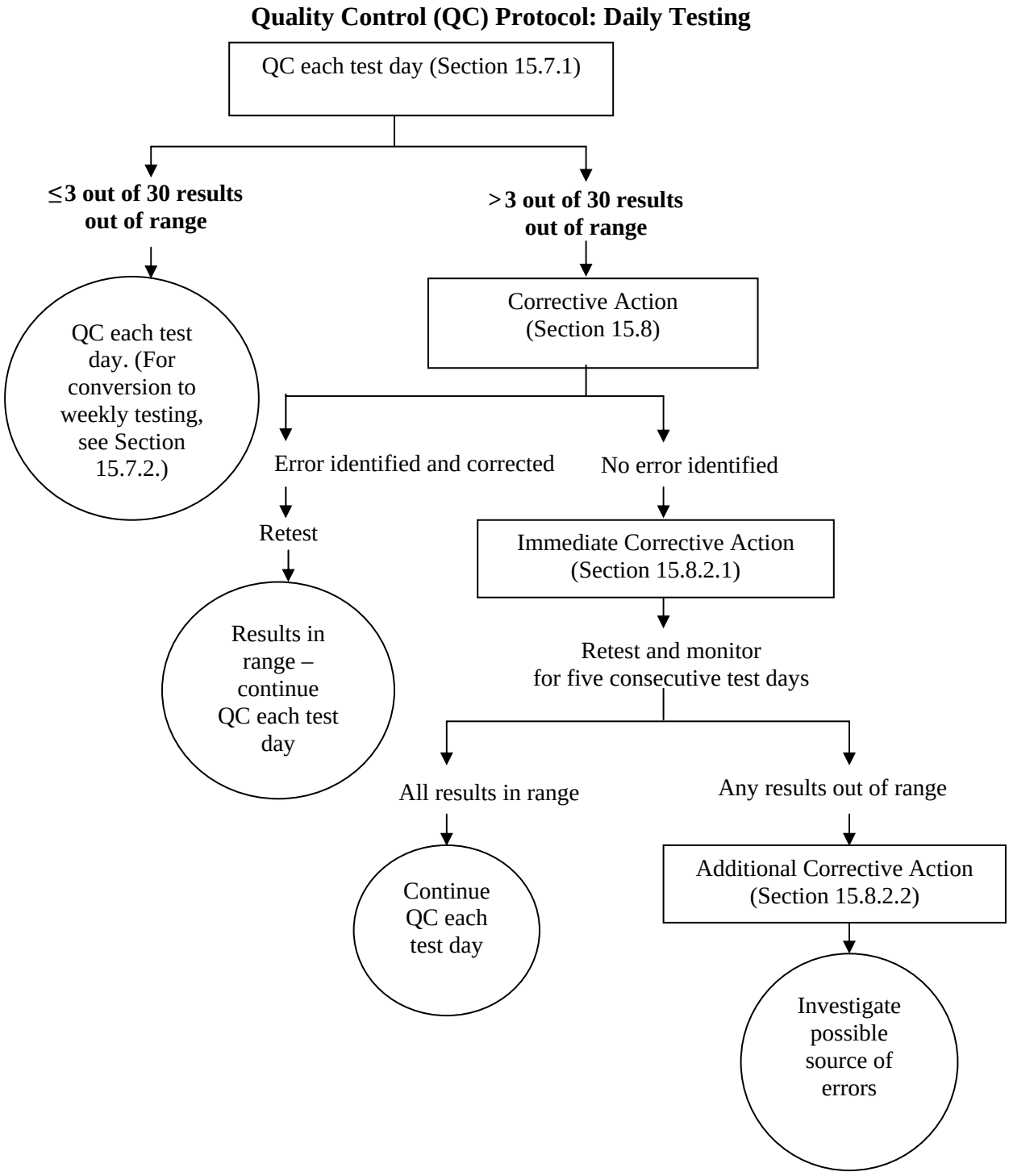
## Appendix A. Quality Control Protocol Flow Charts

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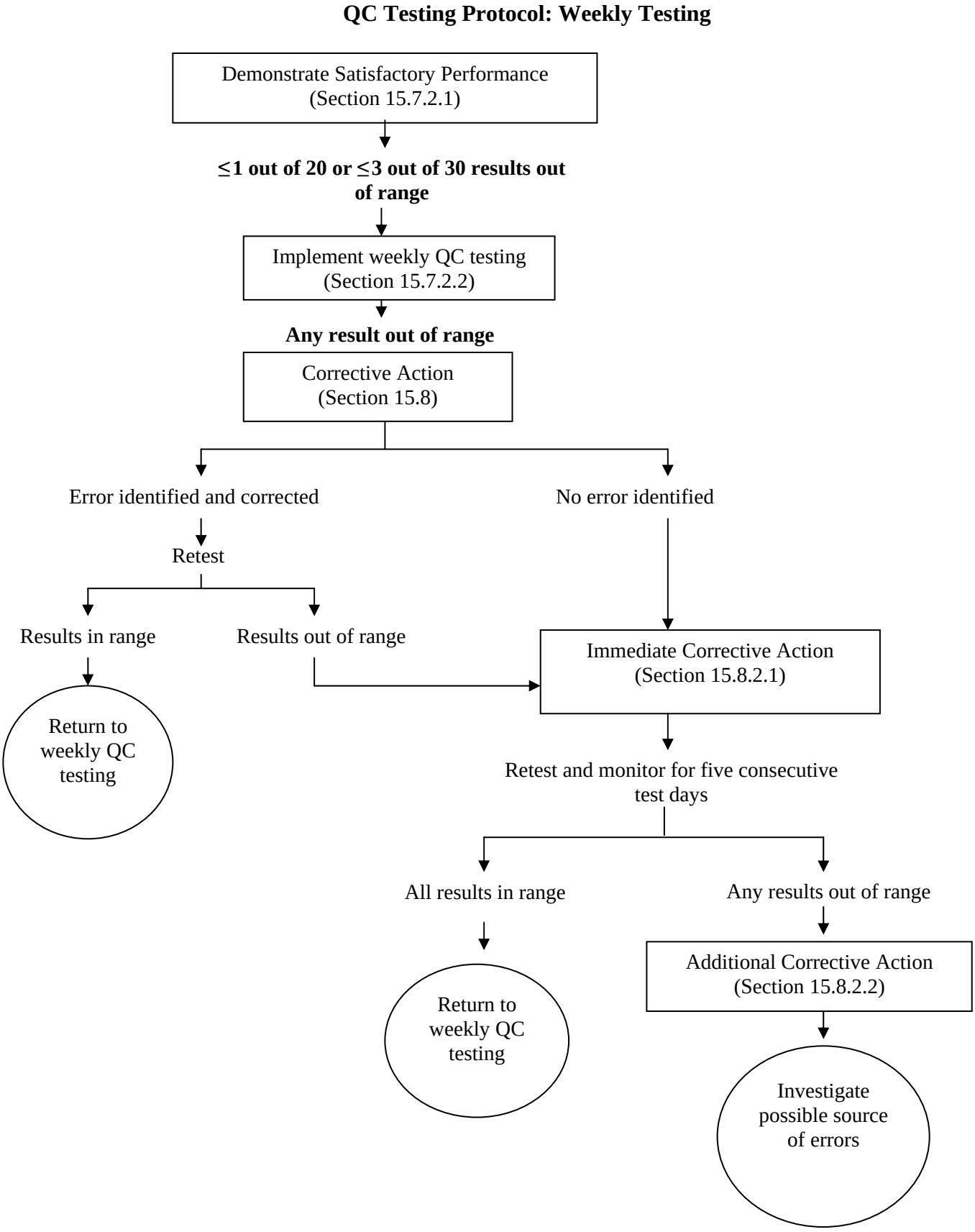
## Appendix A. (Continued)





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# Appendix B. Preparation of Media and Reagents

## B1 Agar Media

### B1.1 Mueller-Hinton Agar

Mueller-Hinton agar (MHA) preparation includes the following steps:

1. Prepare MHA from a commercially available dehydrated base according to the manufacturer's instructions.

2. Immediately after autoclaving, allow the agar to cool in a 45 to 50°C water bath.

3. Pour the freshly prepared and cooled medium into glass or plastic, flat-bottomed Petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm. This corresponds to 60 to 70 mL of medium for plates with a diameter of 150 mm and 25 to 30 mL for plates with a diameter of 100 mm.

4. Allow the agar plates to cool further to room temperature and, unless the plates are used the same day, store in a refrigerator (2 to 8°C).

5. Use the plates within seven days after preparation unless adequate precautions, such as wrapping in plastic, are taken to minimize drying of the agar.

6. A representative sample of each batch of plates should be examined for sterility by incubating at 35 ± 2°C for 24 hours or longer.

7. 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 (eg, aminoglycosides, macrolides), whereas other agents may appear to have excessive activity (eg, 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.

8. Do not add supplemental calcium or magnesium cations to MHA.

### B1.2 Mueller-Hinton Agar + 5% Sheep Blood

1. Prepare MHA as described above through Section B1.1 (2). When it has cooled to 45 to 50°C, add 50 mL of defibrinated sheep blood to 1 L of MHA. Continue as described in Section B1.1.

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.

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## Appendix B. (Continued)

### B1.3 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 $B_{12}$
   - 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
   - 25.9 g L-cysteine HCl

2. Prepare 1 L of single strength GC agar base from a commercially available dehydrated base according to the manufacturer's instructions.

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

4. Add 10 mL of 1% defined growth supplement.

### B1.4 *Haemophilus* Test Medium

In its agar form, *Haemophilus* Test Medium (HTM) consists of the following ingredients:

- Mueller-Hinton broth (MHB)
- 15 μg/mL β-NAD
- 15 μg/mL bovine or porcine hematin
- 5 g/L yeast extract

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 MHA 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 MHA.

4. After autoclaving, cool to 45 to 50°C.

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

6. The pH should be 7.2 to 7.4.


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## Appendix B. (Continued)

**NOTE:** *Haemophilus influenzae* (ATCC®[a] 10211) is recommended as a useful additional quality control (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.

### B2 Reagents

#### B2.1 0.5 McFarland Turbidity Standard

1. Prepare a 0.048 mol/L $BaCl_2$ (1.175% w/v $BaCl_2 \bullet 2H_2O$) stock solution.

2. Prepare a 0.18 mol/L (0.36 N) $H_2SO_4$ (1% v/v) stock solution.

3. With constant stirring to maintain a suspension, add 0.5 mL of the $BaCl_2$ solution to 99.5 mL of the $H_2SO_4$ 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.

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 commercially available. When used, they should be mixed by inverting gently (not on a vortex mixer) immediately before use.

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

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# Appendix C. Conditions for Disk Diffusion Antimicrobial Susceptibility Tests

## C1. Conditions for Disk Diffusion Antimicrobial Susceptibility Tests for Nonfastidious Organisms

| Organism/Organism Group | M100[1] Table | Medium | 0.5 McFarland Inoculum | Incubation | Incubation Time | Minimal Quality Control[a] | Comments/Modifications |
|---|---|---|---|---|---|---|---|
| *Enterobacteriaceae* | 2A | MHA | Direct colony suspension in MHB or saline, or growth method | 35 ± 2°C; ambient air | 16 to 18 hours | *Escherichia coli* ATCC®[b] 25922<br>*E. coli* ATCC® 35218 (for β-lactam/β-lactamase inhibitor combinations) | |
| *Pseudomonas aeruginosa* | 2B-1 | MHA | Direct colony suspension in MHB or saline, or growth method | 35 ± 2°C; ambient air | 16 to 18 hours | *E. coli* ATCC® 25922<br>*P. aeruginosa* ATCC® 27853<br>*E. coli* ATCC® 35218 (for β-lactam/β-lactamase inhibitor combinations) | |
| *Acinetobacter* spp. | 2B-2 | MHA | Direct colony suspension in MHB or saline, or growth method | 35 ± 2°C; ambient air | 20 to 24 hours | *E. coli* ATCC® 25922<br>*P. aeruginosa* ATCC® 27853<br>*E. coli* ATCC® 35218 (for β-lactam/β-lactamase inhibitor combinations) | |
| *Burkholderia cepacia* | 2B-3 | MHA | Direct colony suspension in MHB or saline, or growth method | 35 ± 2°C; ambient air | 20 to 24 hours | *E. coli* ATCC® 25922<br>*P. aeruginosa* ATCC® 27853<br>*E. coli* ATCC® 35218 (for β-lactam/β-lactamase inhibitor combinations) | |
| *Stenotrophomonas maltophilia* | 2B-4 | MHA | Direct colony suspension in MHB or saline, or growth method | 35 ± 2°C; ambient air | 20 to 24 hours | *E. coli* ATCC® 25922<br>*P. aeruginosa* ATCC® 27853<br>*E. coli* ATCC® 35218 (for β-lactam/β-lactamase inhibitor combinations) | |
| *Staphylococcus* spp. | 2C | MHA | Direct colony suspension in MHB or saline | 35 ± 2°C; ambient air (Testing at temperatures above 35°C may not detect MRS.) | 16 to 18 hours;<br>24 hours for oxacillin with *S. aureus;* 24 hours for vancomycin with all staphylococci; 24 hours for cefoxitin with coagulase-negative staphylococci | *Staphylococcus aureus* ATCC® 25923<br>*E. coli* ATCC® 35218 (for β-lactam/β-lactamase inhibitor combinations) | Direct colony suspension only. Examine oxacillin, vancomycin, and linezolid zones carefully with transmitted light for small colonies or haze inside the zone of inhibition; any growth = resistance.<br><br>Vancomycin disk diffusion testing is not recommended for coagulase-negative staphylococci. |

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# Appendix C. (Continued)

## C2. Conditions for Disk Diffusion Antimicrobial Susceptibility Tests for Fastidious Organisms

| Organism/Organism Group | M100[1] Table | Medium | 0.5 McFarland Inoculum | Incubation | Incubation Time | Minimal Quality Control[a] | Comments/Modifications |
|---|---|---|---|---|---|---|---|
| *Enterococcus* spp. | 2D | MHA | Direct colony suspension in MHB or saline, or growth method | 35 ± 2°C; ambient air | 16 to 18 hours; 24 hours for vancomycin | *S. aureus* ATCC® 25923 | Examine vancomycin zones carefully with transmitted light for small colonies or haze inside the zone of inhibition; any growth = resistance. |
| *Haemophilus influenzae* *Haemophilus parainfluenzae* | 2E | HTM | Direct colony suspension in MHB or saline prepared from an overnight (preferably 20- to 24-hour) chocolate agar plate[c] | 35 ± 2°C; 5% $CO_2$ | 16 to 18 hours | *H. influenzae* ATCC® 49247 *H. influenzae* ATCC® 49766 *E. coli* ATCC® 35218 (for amoxicillin-clavulanic acid) | Test a maximum of 9 disks on a 150-mm plate and 4 disks on a 100-mm plate. |
| *Neisseria gonorrhoeae* | 2F | GC agar base with 1% defined supplement | Direct colony suspension in MHB or 0.9% phosphate-buffered saline, pH 7.0, prepared from overnight chocolate agar plate incubated in 5% $CO_2$ | 36 ± 1°C (do not exceed 37°C); 5% $CO_2$ | 20 to 24 hours | *N. gonorrhoeae* ATCC® 49226 | Test a maximum of 9 disks on a 150-mm plate and 4 disks on a 100-mm plate. For some agents, eg, fluoroquinolones or cephalosporins, only 2 to 3 disks may be tested per plate. |
| *Streptococcus pneumoniae* | 2G | MHA with 5% sheep blood | Direct colony suspension in MHB or saline using colonies from an overnight (18- to 20-hour) sheep blood agar plate | 35 ± 2°C; 5% $CO_2$ | 20 to 24 hours | *S. pneumoniae* ATCC® 49619 | Test a maximum of 9 disks on a 150-mm plate and 4 disks on a 100-mm plate. Measure the zone of growth inhibition, not the zone of inhibition of hemolysis. |
| *Streptococcus* spp. | 2H-1 2H-2 | MHA with 5% sheep blood | Direct colony suspension in MHB or saline | 35 ± 2°C; 5% $CO_2$ | 20 to 24 hours | *S. pneumoniae* ATCC® 49619 | Test a maximum of 9 disks on a 150-mm plate and 4 disks on a 100-mm plate. Measure the zone of growth inhibition, not the zone of inhibition of hemolysis. |
| *Neisseria meningitidis* | 2I | MHA with 5% sheep blood[c] | Direct colony suspension in MHB or saline prepared from a 20- to 24-hour chocolate agar plate incubated in 5% $CO_2$[d] | 35 ± 2°C; 5% $CO_2$ | 20 to 24 hours | *S. pneumoniae* ATCC® 49619 (5% $CO_2$) *E. coli* ATCC® 25922 (ambient air or 5% $CO_2$; for ciprofloxacin, nalidixic acid, minocycline, sulfisoxazole) | Test a maximum of 5 disks on a 150-mm plate and 2 disks on a 100-mm plate.[e] Caution: Perform all testing in a BSC. |

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# Appendix C. (Continued)

Abbreviations: BSC, biological safety cabinet; HTM, *Haemophilus* Test Medium; MHA, Mueller-Hinton agar; MHB, Mueller-Hinton broth; MRS, methicillin-resistant staphylococci.

**Footnotes**

[a] See specific M100[1] Supplemental Tables for additional quality control recommendations for screening and confirmatory tests.
[b] ATCC® is a registered trademark of the American Type Culture Collection.
[c] This suspension will contain approximately 1 to $4 \times 10^8$ colony-forming units (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.
[d] Enriched chocolate agar is not recommended for susceptibility testing of *N. meningitidis*.
[e] 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 considered when preparing the final dilution before plate inoculation, as guided by colony counts.

## Reference for Appendix C

[1] CLSI. *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement.* CLSI document M100-S22. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.

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## Appendix D. Quality Control Strains for Antimicrobial Susceptibility Tests (refer to current edition of M100[1] for the most updated version of this table)

| Quality Control Strain | Organism Characteristics | Disk Diffusion Tests | MIC Tests | Screening Tests | Other |
|---|---|---|---|---|---|
| *Enterococcus faecalis* ATCC®[a] 51299 | •Resistant to vancomycin (*VanB*) and high-level aminoglycosides | | | •Vancomycin agar<br>•High-level aminoglycoside resistance | |
| *Escherichia coli* ATCC® 25922 | •β-Lactamase negative | •Nonfastidious gram-negatives<br>•*Neisseria meningitidis* | •Nonfastidious gram-negatives<br>•*N. meningitidis* | | |
| *E. coli* ATCC® 35218 | •Contains plasmid-encoded TEM-1 β-lactamase (non-ESBL)[b,c,f,g] | •β-Lactam/β-lactamase inhibitor combinations | •β-Lactam/β-lactamase inhibitor combinations | | |
| *Haemophilus influenzae* ATCC® 49247 | •BLNAR | •*Haemophilus* spp. | •*Haemophilus* spp. | | |
| *H. influenzae* ATCC® 49766 | •Ampicillin susceptible | •*Haemophilus* spp. (more reproducible with selected β-lactams) | •*Haemophilus* spp. (more reproducible with selected β-lactams) | | |
| *Klebsiella pneumoniae* ATCC® 700603 | •Contains SHV-18 ESBL[c,f,g] | •ESBL screen and confirmatory tests | •ESBL screen and confirmatory tests | | |
| *Neisseria gonorrhoeae* ATCC® 49226 | • CMRNG | •*N. gonorrhoeae* | •*N. gonorrhoeae* | | |
| *Pseudomonas aeruginosa* ATCC® 27853[d] | • Contains inducible AmpC β-lactamase | •Nonfastidious gram-negatives | •Nonfastidious gram-negatives | | •Assess suitability of cation content in each batch/lot of Mueller-Hinton for gentamicin MIC and disk diffusion. |
| *Staphylococcus aureus* ATCC® 25923 | •β-Lactamase negative<br>•*mec*A negative<br>•Little value in MIC testing due to extreme susceptibility to most drugs | •Nonfastidious gram-positives | | | |

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## Appendix D. (Continued)

| Quality Control Strain | Organism Characteristics | Disk Diffusion Tests | MIC Tests | Screening Tests | Other |
|---|---|---|---|---|---|
| *S. aureus* ATCC® 29213 | • Weak β-lactamase–producing strain<br>• *mec*A negative | | • Nonfastidious gram-positives | • Oxacillin agar | |
| *S. aureus* ATCC® 43300 | • Oxacillin-resistant, *mec*A positive | • Cefoxitin disk diffusion testing | • Cefoxitin MIC testing | • Oxacillin agar | |
| *S. 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 | • *S. pneumoniae*<br>• *Streptococcus* spp.<br>• *N. meningitidis* | • *S. pneumoniae*<br>• *Streptococcus* spp.<br>• *N. meningitidis* | | |
| **Supplemental QC Strains**[h] | | | | | |
| *E. faecalis* ATCC® 29212 | | | • Ceftaroline MIC testing | | |
| *E. faecalis* ATCC® 33186 | | | | | • Alternative to *E. faecalis* ATCC® 29212 to assess suitability of medium for sulfonamide or trimethoprim MIC tests[e] |
| *H. influenzae* ATCC® 10211 | | | | | • Assess each batch/lot for growth capabilities of HTM. |
| *K. pneumoniae* ATCC® BAA-1705 | • KPC-producing strain[c]<br>• MHT positive | • Phenotypic confirmatory test for carbapenemase production (MHT) | | | |
| *K. pneumoniae* ATCC® BAA-1706 | • Resistant to carbapenems by mechanisms other than carbapenemase<br>• MHT negative | • Phenotypic confirmatory test for carbapenemase production (MHT) | | | |
| *S. 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[1] Tables 2C Supplemental Tables 2 and 3, and Tables 3A and 4A | | |
| *S. 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[1] Table 2C Supplemental Tables 2 and 3, and Tables 3A and 4A | | |

Abbreviations: BLNAR, β-lactamase negative, ampicillin resistant; CMRNG, chromosomally mediated penicillin-resistant *Neisseria gonorrhoeae*; ESBL, extended-spectrum β-lactamase; HLAR, high-level aminoglycoside resistance; HTM, *Haemophilus* Test Medium; KPC, *Klebsiella pneumoniae* carbapenemase; MHT, modified Hodge test; MIC, minimal inhibitory concentration; QC, quality control; TEM, Temoneira (first patient from whom a TEM β-lactamase–producing strain was reported).

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# Appendix D. (Continued)

**Footnotes**

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

[b] *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-clavulanic acid). 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.

[c] 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, ticarcillin), decreased MICs for *K. pneumoniae* ATCC® 700603 with cephalosporins and aztreonam, and false-negative MHT with *K. pneumoniae* ATCC® BAA-1705.

[d] 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.

[e] 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.

[f] Rasheed JK, Anderson GJ, Yigit H, et al. Characterization of the extended-spectrum beta-lactamase reference strain, *Klebsiella pneumoniae* K6 (ATCC® 700603), which produces the novel enzyme SHV-18. *Antimicrob Agents Chemother.* 2000;44(9):2382-2388.

[g] Queenan AM, Foleno B, Gownley C, Wira E, Bush K. Effects of inoculum and beta-lactamase activity in AmpC- and extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates tested by using NCCLS ESBL methodology. *J Clin Microbiol.* 2004;42(1):269-275.

[h] See Section 15.3.

## Reference for Appendix D

[1] CLSI. *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement.* CLSI document M100-S22. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.

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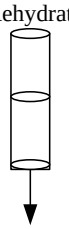
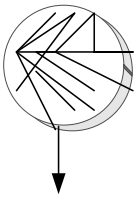
## Appendix E. Quality Control Strain Maintenance

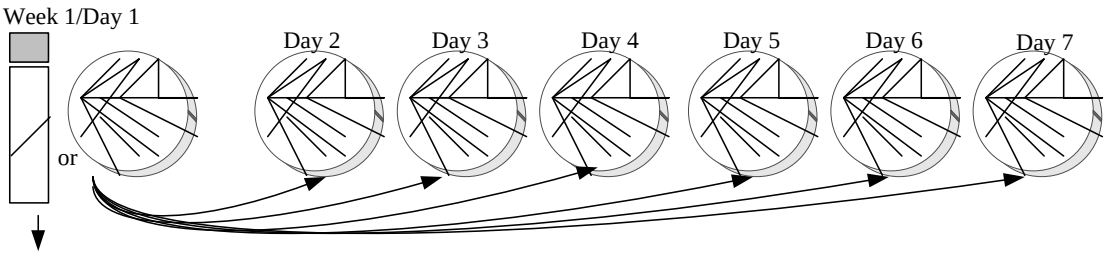
1. Rehydrate new stock culture or obtain strain from frozen stock.



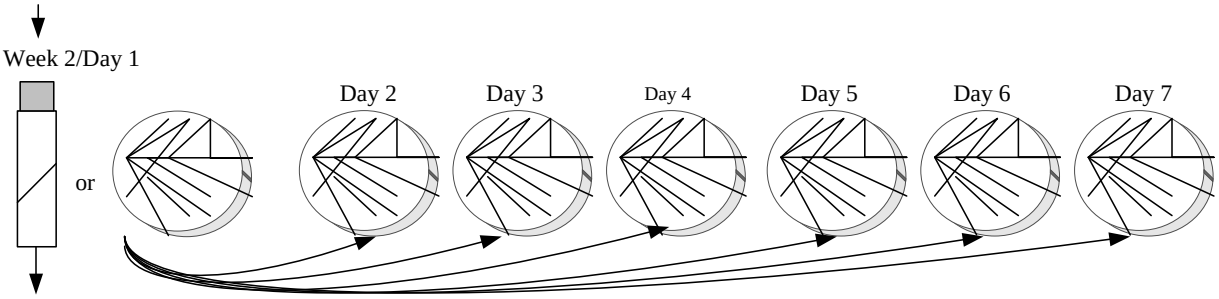
2. Subculture to appropriate media and incubate (primary subculture).



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 quality control 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, *Pseudomonas aeruginosa* ATCC®[a] 27853, *Enterococcus faecalis* ATCC® 51299, *Streptococcus pneumoniae* ATCC® 49619).

[a] ATCC® is a registered trademark of the American Type Culture Collection.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## The Quality Management System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management system approach in the development of standards and guidelines, which facilitates project management; defines a document structure via a template; and provides a process to identify needed documents. The quality management system approach applies a core set of "quality system essentials" (QSEs), basic to any organization, to all operations in any health care service's path of workflow (ie, operational aspects that define how a particular product or service is provided). The QSEs provide the framework for delivery of any type of product or service, serving as a manager's guide. The QSEs are as follows:

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

M02-A11 addresses the QSE indicated by an "X." For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

| Organization | Customer Focus | Facilities and Safety | Personnel | Purchasing and Inventory | Equipment | Process Management | Documents and Records | Information Management | Nonconforming Event Management | Assessments | Continual Improvement |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | M29 | | | | X<br>M06<br>M07<br>M11<br>M23<br>M45 | M07 | | | | |

### Path of Workflow

A path of workflow is the description of the necessary processes to deliver the particular product or service that the organization or entity provides. A laboratory path of workflow consists of the sequential processes: preexamination, examination, and postexamination and their respective sequential subprocesses. All laboratories follow these processes to deliver the laboratory's services, namely quality laboratory information.

M02-A11 addresses the clinical laboratory path of workflow processes 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.

| **Preexamination** | | | | **Examination** | | | **Postexamination** | |
|---|---|---|---|---|---|---|---|---|
| Examination ordering | Sample collection | Sample transport | Sample receipt/processing | Examination | Results review and follow-up | Interpretation | Results reporting and archiving | Sample management |
| | | | | X<br>M07 | X<br>M07<br>M11<br>M100 | X<br>M07<br>M11<br>M100 | X<br>M07<br>M11<br>M100 | |


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## Related CLSI Reference Materials*

**M06-A2** **Protocols for Evaluating Dehydrated Mueller-Hinton Agar; Approved Standard—Second Edition (2006).** This document provides procedures for evaluating production lots of dehydrated Mueller-Hinton agar, and for developing and applying reference media.

**M07-A9** **Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Ninth Edition (2012).** This document addresses reference methods for the determination of minimal inhibitory concentrations of aerobic bacteria by broth macrodilution, broth microdilution, and agar dilution.

**M11-A7** **Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard—Seventh Edition (2007).** This standard 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 Occupationally Acquired Infections; Approved Guideline—Third Edition (2005).** Based on US regulations, this document provides guidance on the risk of transmission of infectious agents by aerosols, droplets, blood, and body substances in a laboratory setting; specific precautions for preventing the laboratory transmission of microbial infection from laboratory instruments and materials; and recommendations for the management of exposure to infectious agents.

**M45-A2** **Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Approved Guideline—Second Edition (2010).** 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 or M07. 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-S22** **Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement (2012).** This document provides updated tables for the Clinical and Laboratory Standards Institute antimicrobial susceptibility testing standards M02-A11 and M07-A9.

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

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Roche Molecular Systems
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Sarstedt, Inc.
Sekisui Diagnostics
Seventh Sense Biosystems
Siemens Healthcare Diagnostics Inc.
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Sysmex Corporation - Japan
Tandem Diagnostics
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The Clinical Microbiology Institute
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TheraDoc
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Theravance Inc.
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Thermo Fisher Scientific, Oxoid Products
Thermo Fisher Scientific, Remel
Transasia Bio-Medicals Limited
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customerservice@clsi.org ▾ www.clsi.org ▾ ISBN 1-56238-781-2 (Print) ▾ ISBN 1-56238-782-0 (Electronic)