

M7-A7  
Vol. 26 No. 2  
Replaces M7-A6  
Vol. 23 No. 2

January 2006

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

This document addresses reference methods for the determination of minimal inhibitory concentrations (MICs) of aerobic bacteria by broth macrodilution, broth microdilution, and agar dilution.

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M7-A7

ISBN 1-56238-587-9

ISSN 0273-3099

Volume 26 Number 2

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### Abstract

Susceptibility testing is indicated for any organism that contributes to an infectious process warranting antimicrobial chemotherapy, if its susceptibility cannot be reliably predicted from knowledge of the organism's identity. Susceptibility tests are most often indicated when the causative organism is thought to belong to a species capable of exhibiting resistance to commonly used antimicrobial agents.

A variety of laboratory methods can be used to measure the *in vitro* susceptibility of bacteria to antimicrobial agents. This document describes standard broth dilution (macrodilution and microdilution) and agar dilution techniques, and it includes a series of procedures to standardize the way the tests are performed. The performance, applications, and limitations of the current CLSI-recommended methods are also described.

The supplemental information (M100 tables) presented with this standard represents the most current information for drug selection, interpretation, and quality control using the procedures standardized in M7. 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-S15) appear in boldface type. Additionally, a glossary of antibiotic terms, classes, and abbreviations (for use with antimicrobial susceptibility *in vitro* test devices) has been added to the tables. It is anticipated that this glossary will provide an additional informational resource to users of the M7 standard, as it outlines antimicrobial terminology used in the susceptibility testing documents that may be unfamiliar or confusing to laboratory users. The glossary, as part of the M100 tabular information, will be continuously updated in future (yearly) revisions of M100.

Clinical and Laboratory Standards Institute. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Seventh Edition*. CLSI document M7-A7 (ISBN 1-56238-587-9). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2006.



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### **Suggested Citation**

(Clinical and Laboratory Standards Institute. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Seventh Edition*. Clinical and Laboratory Standards Institute document M7-A7 [ISBN 1-56238-587-9]. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2006.)

#### **Proposed Standard**

June 1980

#### **Tentative Standard**

December 1982

#### **Approved Standard**

June 1986

#### **Tentative Standard—Second Edition**

November 1988

#### **Approved Standard—Second Edition**

April 1990

#### **Approved Standard—Third Edition**

December 1993

#### **Approved Standard—Fourth Edition**

January 1997

#### **Approved Standard—Fifth Edition**

January 2000

#### **Approved Standard—Sixth Edition**

January 2003

#### **Approved Standard—Seventh Edition**

January 2006

ISBN 1-56238-587-9

ISSN 0273-3099

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## Contents

Abstract.....	i
Committee Membership.....	iii
Foreword.....	vii
Summary of Major Changes in This Document .....	vii
Subcommittee on Antimicrobial Susceptibility Testing Mission Statement .....	ix
1 Scope.....	1
2 Introduction.....	1
3 Standard Precautions.....	1
4 Definitions .....	2
5 Indications for Performing Susceptibility Tests.....	2
6 Antimicrobial Agents.....	3
6.1 Source .....	3
6.2 Weighing Antimicrobial Powders.....	4
6.3 Preparing Stock Solutions.....	5
6.4 Number of Concentrations Tested .....	5
7 Selection of Antimicrobial Agents for Routine Testing and Reporting.....	5
7.1 Routine Reports .....	6
7.2 Nonproprietary Names.....	6
7.3 Selection Guidelines .....	8
7.4 Suggested Guidelines for Routine and Selective Testing and Reporting .....	9
8 Inoculum Preparation for Dilution Tests .....	9
8.1 Turbidity Standard for Inoculum Preparation.....	9
8.2 Direct Colony Suspension Method .....	10
8.3 Growth Method.....	10
9 Agar Dilution Procedure .....	11
9.1 Reagents and Materials.....	11
9.2 Preparing Agar Dilution Plates .....	12
9.3 Preparing the Inoculum.....	13
9.4 Inoculating Agar Dilution Plates .....	13
9.5 Incubating Agar Dilution Plates.....	14
9.6 Determining Agar Dilution End Points.....	14
10 Broth Dilution Procedures (Macrodilution and Microdilution).....	14
10.1 Mueller-Hinton Broth .....	14
10.2 Preparing and Storing Diluted Antimicrobial Agents.....	16
10.3 Broth Dilution Testing.....	16
11 Fastidious Organisms.....	18

**Contents (Continued)**

11.1	<i>Haemophilus influenzae</i> and <i>H. parainfluenzae</i> .....	18
11.2	<i>Neisseria gonorrhoeae</i> .....	19
11.3	<i>Neisseria meningitidis</i> .....	20
11.4	<i>Streptococcus pneumoniae</i> and Other <i>Streptococcus</i> spp.....	21
12	Problem Organisms.....	21
12.1	Staphylococci.....	21
12.2	Enterococci .....	24
12.3	Extended-Spectrum, $\beta$ -Lactamase-Producing, Gram-Negative Bacilli.....	25
13	Inducible Clindamycin Resistance.....	25
14	$\beta$ -Lactamase Tests .....	26
14.1	Purpose .....	26
14.2	Selecting a $\beta$ -Lactamase Test .....	26
15	Reporting of MIC Results.....	26
15.1	Susceptible .....	27
15.2	Intermediate .....	27
15.3	Resistant.....	27
16	Quality Control Procedures.....	27
16.1	Purpose .....	27
16.2	Quality Control Responsibilities.....	27
16.3	Reference Strains for Quality Control .....	28
16.4	Storing and Testing Quality Control Strains.....	30
16.5	Batch or Lot Quality Control .....	30
16.6	MIC Quality Control Limits .....	31
16.7	Frequency of Quality Control Testing.....	31
16.8	Frequency of Quality Control Testing for Screening Tests .....	32
16.9	Corrective Action.....	32
16.10	Reporting Patient Results When Out-of-Control Tests Occur.....	33
16.11	Verification of Patient Test Results .....	34
16.12	Other Control Procedures .....	34
17	Limitations of Dilution Test Methods.....	35
17.1	Application to Various Organism Groups .....	35
17.2	Misleading Results.....	35
17.3	Emergence of Resistance .....	36
18	Screening Tests .....	36
	References.....	37
	Appendix A. Quality Control Protocol Flow Charts.....	39
	Summary of Comments and Subcommittee Responses.....	41
	Summary of Delegate Comments and Subcommittee Responses.....	44
	The Quality System Approach.....	48
	Related CLSI/NCCLS Publications.....	49

## Foreword

In this 2006 revision of the CLSI document M7—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*, several sections have been added or revised. In particular, a new section on broth microdilution and agar dilution susceptibility testing of *Neisseria meningitidis* that relates to a new [Table 2L in M100](#) has been added. The Problem Organisms section now details a procedure for oxacillin salt-agar screening for the detection of MRSA and a procedure for disk approximation testing for detecting inducible clindamycin resistance for *S. aureus*, coagulase-negative *Staphylococcus* spp., and beta-hemolytic streptococci. The latest version of the M100 tables ([M100-S16](#)) published as an annual volume is made available within this document to ensure that users are aware of the latest subcommittee guidelines related to both methods and the tabular information that normally is presented in the annual tables. [M100-S16](#) will be updated during subcommittee meetings in 2006 and published again as a separate document in January of 2007.

There are many other editorial and procedural changes in this edition of M7 that have resulted from meetings of the Subcommittee on Antimicrobial Susceptibility Testing since 2003. Specific changes for the M100 tables are summarized at the beginning of the [M100-S16](#) document. The most important changes in the M7 document are summarized below.

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

Matthew A. Wikler, MD, MBA, FIDSA  
*Chairholder, Subcommittee on Antimicrobial Susceptibility Testing*

## Summary of Major Changes in This Document

### Additions/Changes

#### **Antimicrobial agents:**

Penem antimicrobial drug class described ([Section 7.2.1.4](#))

Teicoplanin added to glycopeptide drug class ([Section 7.2.2](#))

Lipopeptide antimicrobial drug class described ([Section 7.2.8](#))

Telithromycin and tigecycline added as single-drug class antimicrobial agents ([Section 7.2.9](#))

#### **Inoculum Preparation:**

The absorbance reading for 0.5 McFarland standard modified ([Section 8.1](#))

Recommendation for use of the direct colony suspension method vs. the growth method for inoculum preparation expanded ([Section 8.2](#))

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

### Reagents:

Problems specified that can occur if pH of Mueller-Hinton agar is outside acceptable range ([Section 9.1.1 \[3\]](#))

The effect that the calcium content of Mueller-Hinton broth can have on daptomycin results outlined ([Section 10.1 \[3\]](#))

Instructions for adding polysorbate 80 for testing dalbavancin specified ([Section 10.1 \[6\]](#))

Alternative instructions for supplementing Mueller-Hinton broth with lysed horse blood added ([Section 10.1 \[4\]](#))

### Fastidious Organisms:

Species included in *Haemophilus* spp. organism group modified ([Section 11.1](#))

Recommendations for broth microdilution and agar dilution susceptibility testing of *Neisseria meningitidis* added ([Section 11.3](#))

### Problem Organisms:

Recommendations for testing and reporting of *mecA* resistance for staphylococcal isolates expanded ([Sections 12.1.1.1, 12.1.1.2, 12.1.1.4](#))

Recommendations for the vancomycin agar BHI screen for *Staphylococcus aureus* added ([Section 12.1.4](#))

Testing oxacillin-resistant *Staphylococcus lugdunensis* detailed ([Section 12.1.1.1](#))

Use of cefoxitin disk as a surrogate for detecting oxacillin resistance in staphylococci described ([Section 12.1.1.2](#))

Recommendations for the vancomycin agar screen for enterococci modified ([Section 12.2.3](#))

### Inducible Clindamycin Resistance:

Recommendations for detecting inducible clindamycin resistance in *Staphylococcus* spp. and beta-hemolytic streptococci added ([Section 13](#))

### Quality Control:

Control strains for oxacillin salt-agar screen added ([Section 16.3](#))

Recommendations to test for the presence of plasmid for QC strain *E. coli* ATCC® 35218 added ([Section 16.3](#))

QC recommendations for monitoring thymidine levels in Mueller-Hinton agar when testing trimethoprim and sulfonamides added ([Section 16.3](#))

Frequency options for QC clarified ([Section 16.7](#))

Incubation temperature range clarified throughout document

### Application of Dilution Test Methods

List expanded to include potential agents of bioterrorism ([Section 17.1](#))

**It is important for users of M2-A9 and M7-A7 to recognize that commercial susceptibility testing devices are not addressed in these standards. The methods described herein are generic reference procedures that can be used for routine susceptibility testing by clinical laboratories, or that can be used by clinical laboratories to evaluate commercial devices for possible routine use. Results generated by the CLSI reference methods are used by the United States Food and Drug Administration to evaluate the performance of commercial systems before clearance is given for marketing in the United States. Clearance by the FDA indicates that the agency concludes that commercial devices provide susceptibility results that are substantially equivalent to results generated using the CLSI reference methods for the organisms and antimicrobial agents described in the manufacturer's approved package insert. Some laboratories could find that a commercial dilution, antibiotic gradient, colorimetric, turbidimetric, fluorometric, or other method is suitable for selective or routine use.**

## **Subcommittee on Antimicrobial Susceptibility Testing Mission Statement**

The Subcommittee on Antimicrobial Susceptibility Testing is composed of representatives from the professions, government, and industry, including microbiology laboratories, government agencies, healthcare providers and educators, and pharmaceutical and diagnostic microbiology industries. Using the CLSI voluntary consensus process, the subcommittee develops standards that promote accurate antimicrobial susceptibility testing and appropriate reporting.

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

- develop standard reference methods for antimicrobial susceptibility tests;
- provide quality control parameters for standard test methods;
- establish interpretive criteria for the results of standard antimicrobial susceptibility tests;
- provide suggestions for testing and reporting strategies that are clinically relevant and cost-effective;
- continually refine standards and optimize the detection of emerging resistance mechanisms through the development of new or revised methods, interpretive criteria, and quality control parameters;
- educate users through multimedia communication of standards and guidelines; and
- 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/NCCLS guidelines. The values that guide this mission are quality, accuracy, fairness, timeliness, teamwork, consensus, and trust.

### **Key Words**

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



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

## 1 Scope

This document describes the standard broth (macrodilution and microdilution) and agar dilution methods used to determine the *in vitro* susceptibility of bacteria that grow aerobically. It addresses preparation of broth and agar dilution tests, testing conditions (including inoculum preparation and standardization, incubation time, and incubation temperature), reporting of MIC results, quality control procedures, and limitations of the dilution test methods. To assist the clinical laboratory, suggestions are provided on the selection of antimicrobial agents for routine testing and reporting. Standards for testing the *in vitro* susceptibility of bacteria that grow aerobically utilizing the antimicrobial disk susceptibility testing method are found in CLSI document [M2—Performance Standards for Antimicrobial Disk Susceptibility Tests](#). Standards for testing the *in vitro* susceptibility of bacteria that grow anaerobically are found in CLSI/NCCLS document [M11—Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria](#).

## 2 Introduction

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

This document describes reference standard broth dilution (macrodilution and microdilution) and agar dilution methods. The basics of these methods are derived, in large part, from information generated by the International Collaborative Study.<sup>1</sup> Although these methods are standard reference methods, some are sufficiently practical for routine use in both clinical laboratories and research laboratories.

Commercial systems based primarily, or in part, on certain of these methods are available and may provide essentially equivalent results to the CLSI methods described here. The United States Food and Drug Administration (FDA) is responsible for the approval of commercial devices used in the United States. CLSI does not approve or endorse commercial products or devices. The methods described in this document are intended primarily for testing commonly isolated aerobic or facultative bacteria that grow well after overnight incubation in unsupplemented Mueller-Hinton agar or broth. Alternative media and methods for some fastidious or uncommon organisms are described in [Section 11](#) and [Tables 2E through 2L](#) and [Table 7](#). Methods for testing anaerobic bacteria are outlined in CLSI/NCCLS document [M11—Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria](#).

This document describes methods, quality control, and interpretive criteria recommended presently for dilution susceptibility tests. When new problems are recognized or improvements in these criteria are developed, changes will be incorporated into future editions of this standard and also distributed in annual informational supplements.

## 3 Standard Precautions

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

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

## 4 Definitions

**antimicrobial susceptibility test interpretive category – 1)** 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; **2) susceptible antimicrobial susceptibility test interpretive category** – a category that implies that isolates are inhibited by the usually achievable concentrations of antimicrobial agent when the recommended dosage is used for the site of infection; **3) intermediate antimicrobial susceptibility test interpretive category** – the “intermediate” category includes isolates with antimicrobial agent MICs that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates. The intermediate category implies clinical efficacy in body sites where the drugs are physiologically concentrated (e.g., quinolones and  $\beta$ -lactams in urine) or when a higher than normal dosage of a drug can be used (e.g.,  $\beta$ -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; **4) resistant antimicrobial susceptibility test interpretive category** – a category that implies that isolates are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules and/or that demonstrate MICs that fall in the range where specific microbial resistance mechanisms (e.g.,  $\beta$ -lactamases) are likely, and clinical efficacy of that agent against the isolate has not been reliably shown in treatment studies.

**minimal inhibitory concentration (MIC)** – the lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism in an agar or broth dilution susceptibility test.

**quality control** – the operational techniques that are used to ensure accuracy and reproducibility.

## 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 (e.g., 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 for susceptibility. Identification procedures are often performed at the same time. Mixtures of different types of microorganisms should not be tested on the same susceptibility test plate or

panel. The practice of conducting susceptibility tests directly with clinical material (e.g., 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 being treated, susceptibility tests are often unnecessary, and the results may be misleading.

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

MICs have been determined using concentrations derived traditionally from serial twofold dilutions indexed to the base 2 (e.g., 1, 2, 4, 8, 16 µg/mL). Other dilution schemes have also been used, including use of as few as two widely separated or “breakpoint” concentrations or concentrations between the usual values (e.g., 4, 6, 8, 12, 16 µg/mL). The results from these alternative methods may be equally useful clinically; however, some are more difficult to control (see [Section 16.3](#)). When there is inhibition of growth at the lowest concentration tested, the true MIC value cannot be accurately determined and should be reported as equal to or less than the lowest concentration tested. To apply interpretive criteria when concentrations between the usual dilutions are tested, results falling between serial twofold dilutions should be rounded up to the next highest concentration (e.g., an MIC of 6 µg/mL would become 8 µg/mL).

Whenever MIC results are reported to clinicians to direct therapy, an interpretive category (i.e., susceptible, intermediate, or resistant) should accompany the MIC result based on the criteria outlined in [Tables 2A through 2L and Table 7](#). When tests in which four or fewer consecutive concentrations are tested or when nonconsecutive concentrations are tested, an interpretive category result must be reported. The MIC result may also be reported if desired.

## 6 Antimicrobial Agents

### 6.1 Source

Obtain antimicrobial standards or reference powders directly from the drug manufacturer, from the United States Pharmacopoeia ([www.usp.org](http://www.usp.org), 12601 Twinbrook Parkway, Rockville, Maryland 20852, 800-227-8772), or from certain other commercial sources. Parenteral preparations should not be used for susceptibility testing. Acceptable powders bear a label that states the drug's generic name, lot number, potency (usually expressed in micrograms [µg] or International Units [IU] per mg of powder), and expiration date. Store the powders as recommended by the manufacturer or at ≤-20 °C in a desiccator (preferably in a vacuum). When the desiccator is removed from the refrigerator or freezer, allow the desiccator to warm to room temperature before opening to avoid condensation of water.

## 6.2 Weighing Antimicrobial Powders

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

The value for potency supplied by the manufacturer should include consideration of measures of purity (usually by HPLC assay), water content (e.g., by Karl Fischer analysis or by weight loss on drying), and the salt/counter-ion fraction (if the compound is supplied as a salt instead of free acid or base). The potency may be expressed as a percentage, or in units of  $\mu\text{g}/\text{mg}$  (w/w).

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

Example: meropenem trihydrate

Certificate of analysis data:

Assay purity (by HPLC): 99.8%

Measured water content (by Karl Fischer analysis): 12.1% (w/w)

Active fraction: 100% (supplied as the free acid and not a salt)

Potency calculation from above data:

Potency = (Assay purity) x (Active fraction) x (1- Water Content)

Potency = (99.8) x (1.0) x (1- 0.121)

Potency = 87.7  $\mu\text{g}/\text{mg}$  or 87.7%

Use either of the following formulas to determine the amount of powder **or** diluent needed for a standard solution:

$$\text{Weight (mg)} = \frac{\text{Volume (mL)} \cdot \text{Concentration } (\mu\text{g}/\text{mL})}{\text{Potency } (\mu\text{g}/\text{mg})} \quad (1)$$

or

$$\text{Volume (mL)} = \frac{\text{Weight (mg)} \cdot \text{Potency } (\mu\text{g}/\text{mg})}{\text{Concentration } (\mu\text{g}/\text{mL})} \quad (2)$$

Weigh the antimicrobial powder on an analytical balance that has been calibrated with National Institute of Standards and Technology (NIST) weights (or other approved reference weights). If possible, weigh more than 100 mg of powder. It is advisable to accurately weigh a portion of the antimicrobial agent in excess of that required and to calculate the volume of diluent needed to obtain the final concentration desired as in formula 2 above.

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

$$\text{Volume (mL)} = \frac{182.6 \text{ mg} \cdot 750 \text{ } \mu\text{g/mg}}{1280 \text{ } \mu\text{g/mL}} = 107.0 \text{ mL}$$

*(Desired Concentration)*

(3)

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

### 6.3 Preparing Stock Solutions

Antimicrobial agent stock solutions are to be prepared at concentrations of at least 1000  $\mu\text{g/mL}$  (example: 1280  $\mu\text{g/mL}$ ) or ten times the highest concentration to be tested, whichever is greater. Some antimicrobial agents, however, have limited solubility and may require preparation of lower stock concentrations. In all cases, consider directions provided by the drug's manufacturer as part of determining solubility.

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

- Use a minimum amount of solvent to solubilize the antimicrobial powder.
- Finish diluting the final stock concentration with water or the appropriate diluent as indicated in [Table 4](#).
- For potentially toxic solvents, consult the material safety data sheets (MSDS) available from the manufacturer (see [Table 4](#)).

Because microbial contamination is extremely rare, solutions that have been prepared aseptically but not filter sterilized are generally acceptable. If desired, however, solutions may be sterilized by membrane filtration. Paper, asbestos or sintered glass filters, which may adsorb appreciable amounts of certain antimicrobial agents, must *not* be used. Whenever filtration is used, it is important to document the absence of adsorption by appropriate assay procedures.

Dispense small volumes of the sterile stock solutions into sterile glass, polypropylene, polystyrene, or polyethylene vials; carefully seal; and store (preferably at  $-60\text{ }^{\circ}\text{C}$  or below but never at a temperature warmer than  $-20\text{ }^{\circ}\text{C}$  and never in a self-defrosting freezer). Vials may be thawed as needed and used the same day. Any unused stock solution should be discarded at the end of the day. Stock solutions of most antimicrobial agents can be stored at  $-60\text{ }^{\circ}\text{C}$  or below for six months or more without significant loss of activity. In all cases, directions provided by the drug's manufacturer must be considered in addition to these general recommendations. Any significant deterioration of an antimicrobial agent should be reflected in the results of susceptibility testing using quality control strains.

### 6.4 Number of Concentrations Tested

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

## 7 Selection of Antimicrobial Agents for Routine Testing and Reporting

Selection of the most appropriate antimicrobial agents to test and to report is a decision best made by each clinical laboratory in consultation with the infectious disease practitioners and the pharmacy, as well as the pharmacy and therapeutics and infection control committees of the medical staff. The

recommendations in [Tables 1 and 1A](#) for each organism group list agents of proven efficacy that show acceptable *in vitro* test performance. Considerations in the assignment of agents to specific test/report groups include clinical efficacy, prevalence of resistance, minimizing emergence of resistance, cost, FDA clinical indications for usage, and current consensus recommendations for first choice and alternative drugs. Tests of selected agents may be useful for infection control purposes.

## 7.1 Routine Reports

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

## 7.2 Nonproprietary Names

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

### 7.2.1 $\beta$ -Lactams

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

#### 7.2.1.1 Penicillins

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

#### 7.2.1.2 $\beta$ -Lactam/ $\beta$ -Lactamase Inhibitor Combinations

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

#### 7.2.1.3 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, as well as the agents in subclasses cephamycin, oxacephem, and carbacephems (see [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.

#### 7.2.1.4 Penems

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

#### 7.2.1.5 Monobactams

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

### 7.2.2 Glycopeptides

Glycopeptide antimicrobial agents, which include vancomycin and teicoplanin, 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  $\beta$ -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  $\beta$ -lactam-resistant, gram-positive bacterial strains, e.g., methicillin-resistant *Staphylococcus aureus* (MRSA) and some enterococci.

### 7.2.3 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 (e.g., penicillin, ampicillin, and vancomycin) against some resistant, gram-positive bacteria, such as enterococci.

### 7.2.4 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.

### 7.2.5 Tetracyclines

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

### 7.2.6 Quinolones

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

### 7.2.7 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.

### 7.2.8 Lipopeptides

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

### 7.2.9 Single-Drug Classes

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

## 7.3 Selection Guidelines

To make routine susceptibility testing relevant and practical, the number of agents tested should be limited. [Tables 1](#) and [1A](#) of this document list those agents that should 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 boxes in the tables designate clusters of comparable agents that generally need not be duplicated in testing, because interpretive results are usually similar and clinical efficacy is usually comparable. In addition, the word “or” designates a related group of agents which show a nearly identical spectrum of activity and interpretive results and for which cross-resistance and cross-susceptibility are nearly complete. Therefore, usually only one of the agents within each selection box (cluster or related group) need be selected for testing. With few exceptions, the agent reported must be tested, unless reporting based on testing of another agent provides a more accurate result (e.g., susceptibility of staphylococci to cephems based on oxacillin testing). When possible, the agents

tested should match those included in the hospital formulary, or else the report should include footnotes indicating which other nontested agents may have comparable activity.

## 7.4 Suggested Guidelines for Routine and Selective Testing and Reporting

As listed in [Tables 1 and 1A](#), agents in [Group A](#) are considered appropriate for inclusion in a routine, primary testing panel and for routine reporting of results for the specific organism groups.

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

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

[Group U](#) agents (e.g., nitrofurantoin and certain quinolones) 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 (e.g., *Pseudomonas aeruginosa*).

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

[Group Inv.](#) (“investigational”) agents are undergoing clinical investigation for the organism group and have not yet been approved by FDA for use in the United States.

Each laboratory should decide which agents in [Tables 1 and 1A](#) to report routinely ([Group A](#)) and those that might be reported only selectively (from [Group B](#)), in consultation with the infectious diseases practitioners, the pharmacy, as well as the pharmacy and therapeutics and infection control committees of the healthcare institution. Selective reporting should help improve the clinical relevance of test reports and help minimize the selection of multiresistant, healthcare-associated strains by overuse of broad-spectrum agents. Results for [Group B](#) agents not reported routinely should be available on request, or they may be reported for selected specimens. Unexpected resistance, when confirmed, should be reported, e.g., resistance to a secondary agent but susceptibility to a primary agent.

## 8 Inoculum Preparation for Dilution Tests

### 8.1 Turbidity Standard for Inoculum Preparation

To standardize the inoculum density for a susceptibility test, a  $\text{BaSO}_4$  turbidity standard equivalent to a 0.5 McFarland standard or its optical equivalent (e.g., latex particle suspension) should be used. Prepare a  $\text{BaSO}_4$  0.5 McFarland standard as follows:

- (1) Add a 0.5-mL aliquot of 0.048 mol/L  $\text{BaCl}_2$  (1.175% w/v  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ ) to 99.5 mL of 0.18 mol/L (0.36 N)  $\text{H}_2\text{SO}_4$  (1% v/v) with constant stirring to maintain a suspension.

- (2) 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.
- (3) 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.
- (4) Tightly seal the tubes and store in the dark at room temperature.
- (5) 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.
- (6) The barium sulfate standards should be replaced or their densities verified monthly.

## 8.2 Direct Colony Suspension Method

- (1) The direct colony suspension method is the most convenient method for inoculum preparation. This method can be used with most organisms and is the recommended method for some ([see below](#)).
- (2) Prepare the inoculum by making a direct broth or saline suspension of isolated colonies selected from an 18- to 24-hour agar plate (a nonselective medium, such as blood agar, should be used). Adjust the suspension to achieve a turbidity equivalent to a 0.5 McFarland turbidity standard. This results in a suspension containing approximately  $1$  to  $2 \times 10^8$  CFU/mL for *E. coli* ATCC<sup>®a</sup> 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.
- (3) Direct colony suspension is the recommended method for testing the fastidious organisms, *Haemophilus* spp., *N. gonorrhoeae*, *N. meningitidis*, and streptococci (see [Section 11](#)) and for testing staphylococci for potential methicillin or oxacillin resistance.

## 8.3 Growth Method

- (1) 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 non-fastidious organisms (except staphylococci) when fresh (24 hour) colonies, as required for the direct colony suspension method, are not available.
- (2) Select at least three to five well-isolated colonies of the same morphologic type from an agar plate culture. Touch the top of each colony with a loop or sterile swab and transfer the growth into a tube containing 4 to 5 mL of a suitable broth medium, such as tryptic soy broth.
- (3) Incubate the broth culture at 35 °C until it achieves or exceeds the turbidity of the 0.5 McFarland standard (usually two to six hours).
- (4) 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<sup>®</sup> 25922. To perform this step accurately, use either a photometric device or, if performed visually, use adequate light to visually compare the

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<sup>a</sup> ATCC is a registered trademark of the American Type Culture Collection.

inoculum tube and the 0.5 McFarland standard against a card with a white background and contrasting black lines.

## 9 Agar Dilution Procedure

The agar dilution method for determining antimicrobial susceptibility is a well-established technique.<sup>1,2</sup> The antimicrobial agent is incorporated into the agar medium, with each plate containing a different concentration of the agent. The inocula can be applied rapidly and simultaneously to the agar surfaces using an inoculum-replicating apparatus<sup>3</sup> capable of transferring 32 to 36 inocula to each plate.

### 9.1 Reagents and Materials

#### 9.1.1 Mueller-Hinton Agar

Of the many media available, the subcommittee considers Mueller-Hinton agar 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 sulfonamide, trimethoprim, and tetracycline inhibitors.
- It supports satisfactory growth of most pathogens.
- Large bodies of data and experience have been collected about susceptibility tests performed with this medium.

Although Mueller-Hinton agar is generally reliable for susceptibility testing, results obtained with some batches may, on occasion, vary significantly. Only Mueller-Hinton agar formulations that have been tested according to, and that meet the acceptance limits described in CLSI document M6—*Protocols for Evaluating Dehydrated Mueller-Hinton Agar* should be used.

- (1) New lots of medium should be performance tested before use as outlined in [Section 16.5](#).
- (2) Prepare Mueller-Hinton agar from a commercially available dehydrated base as recommended by the manufacturer. Immediately after autoclaving, allow the agar to cool to 45 to 50 °C in a water bath before aseptically adding antimicrobial solutions and heat-labile supplements, and pouring the plates (see [Section 9.2](#)).
- (3) Check the pH of each batch of Mueller-Hinton agar when the medium is prepared. The exact method used will depend largely on the type of equipment available in the laboratory. The agar medium should have a pH between 7.2 and 7.4 at room temperature and must therefore be checked after gelling. If the pH is less than 7.2, certain drugs will appear to lose potency (e.g., aminoglycosides and macrolides), while other agents may appear to have excessive activity (e.g., tetracyclines). If the pH is greater than 7.4, the opposite effects can be expected. Check the pH by one of the following means:
  - Macerate enough agar to submerge the tip of a pH electrode.
  - Allow a small amount of agar to solidify around the tip of a pH electrode in a beaker or cup.
  - Use a surface electrode.

- (4) Do not add supplemental calcium or magnesium cations to Mueller-Hinton agar. NaCl (2% w/v) should be added to the agar for oxacillin, methicillin, or nafcillin testing of staphylococci.
- (5) Mueller-Hinton agar may be supplemented with 5% (v/v) defibrinated sheep blood or lysed horse blood as outlined in [Tables 2H, 3A, and 7](#). Check the pH after aseptic addition of the blood to the autoclaved and cooled medium. The final pH should be the same as unsupplemented Mueller-Hinton agar, pH 7.2 to 7.4.
- (6) The appropriate medium for agar dilution testing of *Neisseria gonorrhoeae* is GC agar base with defined growth supplement.<sup>4</sup>
- (7) Additional information on dilution or special screening tests for some fastidious or problem organisms is presented in [Sections 11, 12, and 18](#) and in [Table 7](#).

### 9.1.2 Inoculum Replicators

Most inoculum replicators currently available transfer 32 to 36 inocula to each plate.<sup>2</sup> Replicators with pins 3 mm in diameter will deliver approximately 2  $\mu\text{L}$  (range 1 to 3  $\mu\text{L}$ ) onto the agar surface. Those with smaller 1-mm pins will deliver tenfold less, approximately 0.1 to 0.2  $\mu\text{L}$ .<sup>5</sup>

## 9.2 Preparing Agar Dilution Plates

### 9.2.1 Procedure

- (1) Add appropriate dilutions of antimicrobial solution to molten test agars that have been allowed to equilibrate in a water bath to 45 to 50 °C.
- (2) Mix the agar and antimicrobial solution thoroughly and pour into petri dishes on a level surface to result in an agar depth of 3 to 4 mm.
- (3) Pour the plates quickly after mixing to prevent cooling and partial solidification in the mixing container. Bubbles should be avoided.
- (4) Allow the agar to solidify at room temperature (see [Section 9.1.1](#) [3] for pH), and use the plates either immediately or store them in sealed plastic bags at 2 to 8 °C for up to five days for reference work, or longer for routine tests. In one study, agar plates containing cefaclor had to be prepared within 48 hours of use because of degradation of the drug, whereas cefamandole remained stable for greater than the recommended five days.<sup>6</sup> In addition to cefaclor, other antimicrobial agents that are particularly labile are ampicillin, methicillin, imipenem, and clavulanic acid.

**NOTE:** Do not assume that all antimicrobial agents will maintain their potency under these storage conditions. The user should evaluate the stability of plates from results obtained with control strains and should develop applicable shelf-life criteria. This information is sometimes available from pharmaceutical manufacturers.

- (5) Allow plates stored at 2 to 8 °C to equilibrate to room temperature before use. Make certain that the agar surface is dry before inoculating the plates. If necessary, place the plates in an incubator or laminar flow hood for approximately 30 minutes with their lids ajar to hasten drying of the agar surface.

### 9.2.2 Dilution Scheme for Reference Method

In general, a scheme in which one part of antimicrobial solution is added to nine parts of liquid agar, such as shown in [Table 5](#), should be used.

### 9.2.3 Quality Control Frequency

Although weekly quality control, as described in this standard, is acceptable for most drug/agar combinations, some may require more frequent testing, e.g., the labile drugs described in [Section 9.2.1](#) above. See [Section 16](#) for further details.

### 9.2.4 Control Plates

Use drug-free plates prepared from the base medium (with or without supplements as indicated in [Section 9.1.1](#)) as growth controls.

## 9.3 Preparing the Inoculum

### 9.3.1 Inoculum Preparation

Prepare a standardized inoculum for the agar dilution method by either growing microorganisms to the turbidity of the 0.5 McFarland standard or suspending colonies directly to achieve the same density as described in [Section 8](#). Preparation of the initial inoculum and final dilution may vary for certain fastidious organisms such as *Helicobacter pylori* (see [Table 2J](#)).

### 9.3.2 Dilution of Inoculum Suspension

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

## 9.4 Inoculating Agar Dilution Plates

- (1) Arrange the tubes containing the adjusted and diluted bacterial suspensions ( $10^7$  CFU/mL) in order in a rack. Place an aliquot of each well-mixed suspension into the corresponding well in the replicator inoculum block.
- (2) Mark the agar plates for orientation of the inoculum spots.
- (3) Apply an aliquot of each inoculum to the agar surface, either with an inocula-replicating device or with standardized loops or pipettes. Appropriate dilution of the inoculum suspension should be made depending on the volume of inoculum delivery so as to obtain a final concentration of  $10^4$  CFU/spot (see [Section 9.3.2](#)).
- (4) Inoculate a growth-control plate (no antimicrobial agent) first and then, starting with the lowest concentration, inoculate the plates containing the different antimicrobial concentrations. Inoculate a second growth control plate last to ensure that there was no contamination or significant antimicrobial carry-over during the inoculation.

- (5) Streak a sample of each inoculum on a suitable nonselective agar plate and incubate overnight to detect mixed cultures and to provide freshly isolated colonies in case retesting proves necessary.

### 9.5 Incubating Agar Dilution Plates

- (1) Allow the inoculated plates to stand at room temperature until the moisture in the inoculum spots has been absorbed into the agar, i.e., until the spots are dry, but no more than 30 minutes. Invert the plates and incubate at  $35 \pm 2$  °C for 16 to 20 hours (see [Section 12](#) for MRS and vancomycin-resistant *Enterococcus* spp.).
- (2) Do not incubate the plates in an atmosphere with increased CO<sub>2</sub> when testing nonfastidious organisms, because the surface pH may be altered. However, incubate *N. gonorrhoeae*, streptococci, and *N. meningitidis* in an atmosphere containing 5% CO<sub>2</sub> (see [Section 11](#) and [Tables 2F, 2H, and 2L](#)). *Helicobacter pylori* should be incubated in a microaerobic atmosphere (see [Table 2J](#)).

### 9.6 Determining Agar Dilution End Points

- (1) Place the plates on a dark, nonreflecting surface to determine the end points. Record the MIC as the lowest concentration of antimicrobial agent that completely inhibits growth, disregarding a single colony or a faint haze caused by the inoculum. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, read the end point at the concentration in which there is 80% or greater reduction in growth as compared to the control.
- (2) If two or more colonies persist in concentrations of the agent beyond an obvious end point, or if there is no growth at lower concentrations but growth at higher concentrations, check the culture purity and repeat the test if required.

## 10 Broth Dilution Procedures (Macrodilution and Microdilution)

### 10.1 Mueller-Hinton Broth

- (1) Mueller-Hinton broth is recommended as the medium of choice for susceptibility testing of commonly isolated, rapidly growing aerobic, or facultative organisms.<sup>1</sup> Mueller-Hinton broth demonstrates good batch-to-batch reproducibility for susceptibility testing; is low in sulfonamide, trimethoprim, and tetracycline inhibitors; and yields satisfactory growth of most pathogens. In addition, a large body of data and experience has been gathered about tests performed with this medium. Mueller-Hinton broth may be supplemented to support the growth of fastidious bacteria. For example, *Haemophilus* Test Medium<sup>7</sup> (HTM) is recommended for testing *Haemophilus* spp. Blood should be added for testing streptococci. Specific recommendations for supplementing Mueller-Hinton broth to support the growth of fastidious or problem organisms are provided in [Sections 11 and 12](#), and in [Tables 2E, 2G, 2H, 2L, and 7](#).
- (2) Monitor the MIC performance and chemical characteristics of Mueller-Hinton broth routinely. Check the pH of each batch of Mueller-Hinton broth with a pH meter after the medium is prepared; the pH should be between 7.2 and 7.4 at room temperature (25 °C).
- (3) Unless Mueller-Hinton broth has the correct concentrations of the divalent cations Ca<sup>++</sup> and Mg<sup>++</sup> (20 to 25 mg of Ca<sup>++</sup>/L [50 mg/L when testing daptomycin] and 10 to 12.5 mg of Mg<sup>++</sup>/L), MICs of aminoglycosides for *P. aeruginosa*, of tetracycline for all bacteria, and of daptomycin for gram-positive organisms will be different from MICs obtained on Mueller-Hinton agar. Some manufacturers provide Mueller-Hinton broth that has already had cation content adjustment. Therefore, the instructions for cation adjustments below should be followed only when the initial Mueller-Hinton broth has been certified by the manufacturer or measured by the user to contain no or

inadequate amounts of  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  when assayed for total divalent cation content by atomic absorption spectrophotometry. Adding excess cations to Mueller-Hinton broth may lead to erroneous results; however, for testing daptomycin the broth must be supplemented to 50 mg/L of  $\text{Ca}^{++}$ .

#### *Cation Adjustment of Mueller-Hinton Broth*

- (a) To prepare a magnesium stock solution, dissolve 8.36 g of  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  in 100 mL of deionized water. This solution contains 10 mg of  $\text{Mg}^{++}$ /mL.
- (b) To prepare a calcium stock solution, dissolve 3.68 g of  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  in 100 mL of deionized water. This solution contains 10 mg of  $\text{Ca}^{++}$ /mL.

Stock cation solutions should be sterilized by membrane filtration and stored at 2 to 8 °C.

- (c) Prepare Mueller-Hinton broth as the manufacturer directs, autoclave, and chill overnight at 2 to 8 °C or in an ice bath before cation addition if it is to be used the same day. Some  $\text{Ca}^{++}$  or  $\text{Mg}^{++}$  is often present in the commercially prepared dehydrated medium. This starting concentration of cations must be accounted for when calculating the amount of  $\text{Ca}^{++}$  or  $\text{Mg}^{++}$  to add to the medium.
  - (d) With stirring, add 0.1 mL of chilled  $\text{Ca}^{++}$  or  $\text{Mg}^{++}$  stock solution per liter of broth for each desired increment of 1 mg/L in the final concentration in the adjusted Mueller-Hinton broth. This medium is called “cation-adjusted Mueller-Hinton broth” (CAMHB). Adjustments of  $\text{Ca}^{++}$  or  $\text{Mg}^{++}$ , or both, are not necessary when Mueller-Hinton broth as received from the manufacturer already contains the correct concentrations (20 to 25 mg of  $\text{Ca}^{++}$ /L [50 mg/L for daptomycin] and 10 to 12.5 mg of  $\text{Mg}^{++}$ /L) of divalent cations.
- (4) CAMHB may be supplemented with 2.5 to 5% (v/v) lysed horse blood (LHB). To prepare LHB, freeze and thaw defibrinated horse blood until the blood is thoroughly lysed (about five to seven freeze-thaw cycles). Aseptically mix equal volumes of the lysed blood and sterile, distilled water (now 50% LHB). To be used in the broth test, the combination of broth and LHB must be clear, and this can be accomplished by centrifuging the 50% LHB at 12 000 x g for 20 minutes. Decant the supernatant and recentrifuge if necessary. Aseptically add appropriate amounts of the 50% LHB to the broth to yield a final concentration of 2.5 to 5% LHB. Check the pH after aseptic addition of the blood to the autoclaved and cooled broth. The blood may be added to the microdilution trays either when they are first dispensed or after they are thawed just prior to inoculation. If added after plate preparation, the blood must be added along with the inoculum so that further dilution of the antimicrobial agent in the tray does not occur (see Section 10.3.1.1) and that the final concentration of LHB in the well is 2.5 to 5%. Alternatively, add 5  $\mu\text{L}$  of 50% LHB to each 100  $\mu\text{L}$  well prior to tray inoculation. This is only acceptable if total volume of liquids added to the well does not exceed 10  $\mu\text{L}$ .
  - (5) MIC performance characteristics of each batch of broth are evaluated using a standard set of quality control organisms (see [Section 16.3](#)). If a new lot of Mueller-Hinton broth does not yield the expected MICs, the cation content should be investigated along with other variables and components of the test.
  - (6) The addition of polysorbate 80 (P-80) to the broth at a final concentration of 0.002% (vol/vol) is required for determination of dalbavancin MICs. Broth containing 0.002% P-80 is prepared by first diluting full strength P-80 50-fold (i.e., 1:50) in water to make a 2% stock solution and then further diluting this 2% solution 1000-fold (1:1000) in broth to make a final concentration of 0.002%.

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

## 10.2 Preparing and Storing Diluted Antimicrobial Agents

### 10.2.1 Macrodilution (Tube) Broth Method

- (1) Use sterile 13- x 100-mm test tubes to conduct the test.
- (2) Use a control tube containing broth without antimicrobial agent for each organism tested.
- (3) The tubes can be closed with loose screw-caps, plastic or metal closure caps, or cotton plugs.
- (4) Prepare the final twofold (or other) dilutions of antimicrobial agent volumetrically in the broth. The schedule in [Tables 6](#) and [6A](#) provides a convenient and reliable procedure for preparing dilutions. A minimum final volume of 1 mL of each dilution is needed for the test. A single pipette can be used for measuring all diluents and then for adding the stock antimicrobial solution to the first tube. A separate pipette should be used for each remaining dilution in that set. Because there will be a 1:2 dilution of the drugs when an equal volume of inoculum is added, the antimicrobial dilutions are often prepared at double the desired final concentration.

### 10.2.2 Broth Microdilution Method

- (1) This method is called “microdilution,” because it involves the use of small volumes of broth dispensed in sterile, plastic microdilution trays that have round or conical bottom wells. Each well should contain 0.1 mL of broth. To prepare microdilution trays, antimicrobial agents may be diluted as described in [Section 10.2.1](#) or in [Tables 6](#) and [6A](#).
- (2) The most convenient method of preparing microdilution trays is by use of a dispensing device and antimicrobial dilutions made in at least 10 mL of broth. These dilutions are used to dispense 0.1 ( $\pm 0.02$ ) mL into each of the 96 wells of a standard tray. If the inoculum is to be added by pipette as described in [Section 10.3.1.2](#), prepare the antimicrobial solutions at twice the desired final concentration, and fill the wells with 0.05 mL instead of 0.1 mL. Each tray should include a growth control well and a sterility (uninoculated) well.
- (3) Seal the filled trays in plastic bags and immediately place in a freezer at  $\leq -20$  °C (preferably at  $\leq -60$  °C) until needed. Although the antimicrobial agents in frozen trays usually remain stable for several months, certain agents (e.g., clavulanic acid and imipenem) are more labile than others and should be stored at  $\leq -60$  °C. Do not store the trays in a self-defrosting freezer. Thawed antimicrobial solutions must not be refrozen; repeated freeze-thaw cycles accelerate the degradation of some antimicrobial agents, particularly  $\beta$ -lactams.

## 10.3 Broth Dilution Testing

### 10.3.1 Inoculum Preparation

#### 10.3.1.1 Macrodilution

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

- (2) Optimally within 15 minutes of preparation, dilute the adjusted inoculum suspension in broth so that, after inoculation, each tube contains approximately  $5 \times 10^5$  CFU/mL. This can be accomplished by diluting the 0.5 McFarland suspension 1:150, resulting in a tube containing approximately  $1 \times 10^6$  CFU/mL. The subsequent 1:2 dilution in step 3 will bring the final inoculum to  $5 \times 10^5$  CFU/mL.
- (3) Within 15 minutes after the inoculum has been standardized as described above, add 1 mL of the adjusted inoculum to each tube containing 1 mL of antimicrobial agent in the dilution series (and a positive control tube containing only broth), and mix. This results in a 1:2 dilution of each antimicrobial concentration and a 1:2 dilution of the inoculum. It is advisable to perform a purity check of the inoculum suspension by subculturing an aliquot onto a nonselective agar plate for simultaneous incubation.

#### 10.3.1.2 Microdilution

- (1) Prepare a standardized inoculum using either the direct colony suspension or growth method as described in [Section 8](#).
- (2) Optimally within 15 minutes of preparation, dilute the adjusted inoculum suspension in water, saline, or broth so that, after inoculation, each well contains approximately  $5 \times 10^5$  CFU/mL. The dilution procedure to obtain this final inoculum varies according to the method of delivery of the inoculum to the individual wells and according to the organism being tested, and it must be calculated for each situation. For microdilution tests, the exact inoculum volume delivered to the wells must be known to make this calculation. For example, if the volume of broth in the well is 0.1 mL and the inoculum volume is 0.005 mL, then the 0.5 McFarland suspension ( $1 \times 10^8$  CFU/mL) should be diluted 1:10 to yield  $10^7$  CFU/mL. When 0.005 mL of this suspension is inoculated into the broth, the final test concentration of bacteria will be approximately  $5 \times 10^5$  CFU/mL (or  $5 \times 10^4$  CFU/well in the microdilution method).
- (3) Within 15 minutes after the inoculum has been standardized as described above, inoculate each well of a microdilution tray using an inoculator device that delivers a volume that does not exceed 10% of the volume in the well (e.g.,  $\leq 10 \mu\text{L}$  of inoculum in 0.1 mL antimicrobial agent solution). Conversely, if a 0.05-mL pipette is used, it results in a 1:2 dilution of the contents of each well (containing 0.05 mL), as with the macrodilution procedure. It is advisable to perform a purity check of the inoculum suspension by subculturing an aliquot onto a nonselective agar plate for simultaneous incubation.
- (4) To prevent drying, seal each tray in a plastic bag, with plastic tape, or with a tight-fitting plastic cover before incubating.

#### 10.3.1.3 Colony Counts of Inoculum Suspensions

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

### 10.3.2 Incubation

- (1) Incubate the inoculated macrodilution tubes or microdilution trays at  $35 \pm 2$  °C for 16 to 20 hours in an ambient air incubator. To maintain the same incubation temperature for all cultures, do not stack microdilution trays more than four high.

- (2) Incubation times may differ for fastidious organisms or some problem organisms with difficult-to-detect resistance; follow specific instructions in [Sections 11](#) and [12](#) or the appropriate table in M100.

### 10.3.3 Determining MIC End Points

The MIC is the lowest concentration of antimicrobial agent that completely inhibits growth of the organism in the tubes or microdilution wells as detected by the unaided eye. Viewing devices intended to facilitate reading microdilution tests and recording of results may be used as long as there is no compromise in the ability to discern growth in the wells. Compare the amount of growth in the wells or tubes containing the antibiotic with the amount of growth in the growth-control wells or tubes (no antimicrobial agent) used in each set of tests when determining the growth end points. For a test to be considered valid, acceptable growth ( $\geq 2$  mm button or definite turbidity) must occur in the growth-control well. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, read the end point at the concentration in which there is  $\geq 80\%$  reduction in growth as compared to the control. When a single skipped well occurs in a microdilution test, read the highest MIC. Do not report results for drugs for which there is more than one skipped well. Generally, microdilution MICs for gram-negative bacilli are the same or one twofold dilution lower than the comparable macrodilution MICs.<sup>8</sup>

## 11 Fastidious Organisms

Mueller-Hinton medium described above for the rapidly growing aerobic pathogens is not adequate for susceptibility testing of fastidious organisms. If MIC tests are performed with fastidious organisms, the medium, quality control procedures, and interpretive criteria must be modified to fit each organism. Dilution tests for *H. influenzae* (using HTM), *N. gonorrhoeae* (using GC agar base medium), and streptococci (using lysed horse blood-supplemented CAMHB) have been shown to be reliable methods; they are described here. In addition, the media and important technical aspects of testing the following organisms are given in the table indicated: *Bacillus anthracis* ([Table 2K](#)), *Burkholderia mallei* and *B. pseudomallei* ([Table 2K](#)), *Campylobacter* spp. ([Table 3A](#)), *Francisella tularensis* ([Table 2K](#)), *H. pylori* ([Table 2J](#)), *Listeria* spp. ([Table 7](#)), *N. meningitidis* ([Table 2L](#)), and *Yersinia pestis* ([Table 2K](#)).

### 11.1 *Haemophilus influenzae* and *H. parainfluenzae*

MIC testing of *Haemophilus influenzae* and *H. parainfluenzae* using *Haemophilus* Test Medium (HTM) has been developed only for broth dilution as described below. When *Haemophilus* spp. is used below, it applies only to these two species. The agar dilution method using HTM has not been studied.

#### 11.1.1 Broth Medium

The medium of choice for broth dilution testing of *Haemophilus* spp. is HTM.<sup>7</sup> HTM consists of

- Mueller-Hinton broth;
- 15  $\mu\text{g}/\text{mL}$   $\beta$ -nicotinamide adenine dinucleotide (NAD);
- 15  $\mu\text{g}/\text{mL}$  bovine hematin;
- 5 g/L yeast extract; and
- 0.2 IU/mL thymidine phosphorylase (if sulfonamides or trimethoprim are to be tested).

To make HTM, first prepare a fresh hematin stock solution by dissolving 50 mg of bovine hematin powder in 100 mL of 0.01 mol/L NaOH with heat and stirring until the powder is thoroughly dissolved.

Add 30 mL of the hematin stock solution to 1 L of Mueller-Hinton broth with 5 g of yeast extract. After autoclaving and cooling, aseptically add cations, if needed, as in CAMHB, and 3 mL of an NAD stock solution (50 mg of NAD dissolved in 10 mL of distilled water and filter sterilized). If sulfonamides or trimethoprim are to be tested, add 0.2 IU thymidine phosphorylase to the medium aseptically. The pH should be 7.2 to 7.4.

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

### 11.1.2 Test Procedure

- (1) Use the direct colony suspension procedure when testing *Haemophilus* spp. Using colonies taken directly from an overnight (preferably 20- to 24-hour) chocolate agar culture plate, prepare a suspension of the test organism in Mueller-Hinton broth or saline. Adjust the suspension with broth or saline using a photometric device to achieve a turbidity equivalent to a 0.5 McFarland standard. Suspensions prepared from a 20- to 24-hour agar plate will contain approximately 1 to 2 x 10<sup>8</sup> CFU/mL, while those prepared from a 16- to 18-hour plate will contain more cells (approximately 3 to 4 x 10<sup>8</sup> CFU/mL). Exercise care in preparing this suspension, taking into account the age of the plate from which the inoculum is prepared; inoculum concentrations higher than 5 x 10<sup>5</sup> CFU/mL may lead to false-resistant results with some β-lactam antimicrobial agents, particularly when β-lactamase-producing strains of *H. influenzae* are tested. Use the suspension for tray inoculation within 15 minutes after adjusting the turbidity.
- (2) Follow the remaining procedure for tray or tube inoculation described in [Section 10.3.1.1](#) (macrodilution) or [Section 10.3.1.2](#) (microdilution).
- (3) Incubate the trays or tubes at 35 ± 2 °C in ambient air for 20 to 24 hours before reading the MICs.

### 11.1.3 MIC Interpretive Criteria

The antimicrobial agents suggested for routine testing of *Haemophilus* spp. are listed in [Table 1A](#). Specific MIC interpretive criteria to be used when testing *Haemophilus* spp. are listed in [Table 2E](#).

## 11.2 *Neisseria gonorrhoeae*

MIC testing of *N. gonorrhoeae* has been developed only for agar dilution using GC agar base and 1% defined growth supplement as described below.

### 11.2.1 Agar Medium

The recommended medium for testing *N. gonorrhoeae* consists of GC agar to which a 1% defined growth supplement (1.1 g L-cysteine, 0.03 g guanine HCL, 3 mg thiamine HCL, 13 mg PABA, 0.01 g B12, 0.1 g cocarboxylase, 0.25 g NAD, 1.0 g adenine, 10 g L-glutamine, 100 g glucose, 0.02 g ferric nitrate [in 1 L H<sub>2</sub>O]) is added after autoclaving. The use of a cysteine-free supplement is required for tests with penems and clavulanate. Cysteine-containing defined growth supplements *do not* significantly alter dilution tests with other drugs.

### 11.2.2 Test Procedure

- (1) Use the direct colony suspension procedure when testing *N. gonorrhoeae*. Using colonies taken directly from an overnight chocolate agar culture plate, prepare a suspension equivalent to that of

the 0.5 McFarland standard in Mueller-Hinton broth or 0.9% phosphate buffered saline, pH 7.0. Use the suspension for plate inoculation within 15 minutes after adjusting the turbidity.

- (2) Follow the agar dilution test procedure steps as described in [Section 9](#) for nonfastidious bacteria.
- (3) Incubate the plates at  $36 \pm 1^\circ\text{C}$  (do not exceed  $37^\circ\text{C}$ ) in an atmosphere of 5%  $\text{CO}_2$  for 20 to 24 hours before reading the MICs.

### 11.2.3 MIC Interpretive Criteria

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

## 11.3 *Neisseria meningitidis*

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

When working with cultures, perform any procedure that potentially generates aerosols in a biological safety cabinet. Consider vaccination of microbiology staff that work with invasive meningococcal isolates, although current vaccines are not protective against all serogroups.

### 11.3.1 Testing Media

Broth microdilution testing of *N. meningitidis* isolates is performed using CAMHB with 2 to 5% lysed horse blood; alternatively, agar dilution can be performed using Mueller-Hinton agar supplemented with 5% defibrinated sheep blood.

### 11.3.2 Test Procedure

- (1) Use the direct colony suspension procedure when testing *N. meningitidis*. Using colonies taken directly from an overnight (20 to 24 hour) enriched chocolate agar plate incubated at  $35 \pm 2^\circ\text{C}$  in 5%  $\text{CO}_2$ , prepare a suspension in saline to achieve a turbidity equivalent to a 0.5 McFarland standard. Use the suspension for tray or plate inoculation within 15 minutes after adjusting the turbidity.
- (2) For broth microdilution testing, follow the remaining procedure described in [Section 10.3.1.2](#).
- (3) For agar dilution testing, follow the procedure described in [Section 9](#) for nonfastidious bacteria.
- (4) Incubate trays or agar plates at  $35 \pm 2^\circ\text{C}$  in 5%  $\text{CO}_2$  for 20 to 24 hours before reading the MICs.

### 11.3.3 MIC Interpretive Criteria

Specific MIC interpretive criteria and quality control organisms to be used when testing *N. meningitidis* are listed in [Table 2L](#).

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

MIC testing of *Streptococcus* spp. using CAMHB with 2.5 to 5% lysed horse blood has been developed only for broth dilution as described below. The agar dilution method has not been validated by CLSI.

### 11.4.1 Broth Medium

The recommended broth for testing *S. pneumoniae* and other streptococci is CAMHB supplemented with 2.5 to 5% lysed horse blood. The method for the preparation of lysed horse blood and details about its addition to the broth for testing are given in [Section 10.1](#).

### 11.4.2 Test Procedure

- (1) Use the direct colony suspension procedure as described in [Section 8.2](#) when testing streptococci. Using colonies taken directly from an overnight (18 to 20 hour) sheep blood agar culture plate, prepare a suspension equivalent to that of the 0.5 McFarland standard in either Mueller-Hinton broth or saline. Use the suspension for tray inoculation within 15 minutes after adjusting the turbidity.
- (2) Follow the remaining broth dilution procedure steps, as described in [Section 10.3.1.1](#) (macrodilution) or [Section 10.3.1.2](#) (microdilution).
- (3) Incubate the trays in ambient air at  $35 \pm 2$  °C for 20 to 24 hours before reading the MICs.
- (4) Inducible clindamycin resistance can be identified in beta-hemolytic streptococci using the method described in [Section 13](#).

### 11.4.3 MIC Interpretive Criteria

The antimicrobial agents suggested for routine testing of pneumococci and other streptococci are indicated in [Table 1A](#). Specific MIC interpretive criteria to be used when testing *S. pneumoniae* and other streptococci are listed in [Tables 2G](#) and [2H](#), respectively.

## 12 Problem Organisms

### 12.1 Staphylococci

#### 12.1.1 Methicillin/Oxacillin Resistance

##### 12.1.1.1 Classification

Historically, resistance to the antistaphylococcal, penicillinase-stable penicillins (e.g., 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, e.g., “MRS,” “methicillin resistance,” or “oxacillin resistance.”

Most resistance to oxacillin in staphylococci is mediated by the *mecA* gene, which directs the production of a supplemental penicillin-binding protein, PBP 2a, and is expressed either homogeneously or heterogeneously. Homogeneous resistance is easily detected with standard testing methods, whereas heterogeneous expression may be more difficult to detect with some methods because only a fraction of

the population (e.g., 1 in 100 000 cells) expresses the resistance phenotype. In the past, the presence of resistance to other classes of agents was an indication of oxacillin resistance. However, some MRSA, such as those found in community-associated infections, are not multiply resistant.

Oxacillin-susceptible, *mecA*-negative strains of *Staphylococcus lugdunensis* exhibit oxacillin MICs in the range of 0.25 to 1 µg/mL, whereas *mecA*-positive strains usually exhibit MICs  $\geq 4$  µg/mL. Therefore, the presence of *mecA*-mediated resistance in *S. lugdunensis* is detected more accurately using the *S. aureus* interpretive criteria than the criteria for coagulase-negative staphylococci. This species is now grouped with *S. aureus* in [Table 2C](#) and should be assumed to be included with *S. aureus* in this section.

#### 12.1.1.2 Methods

- Of the penicillinase-stable penicillins, oxacillin is preferred for *in vitro* testing. Oxacillin susceptibility test results can be applied to the other penicillinase-stable penicillins, i.e., cloxacillin, dicloxacillin, flucloxacillin, methicillin, and nafcillin.
- Tests for *mecA* or the protein produced by *mecA*, the penicillin-binding protein 2a (PBP2a, also called PBP2'), are the most accurate methods for prediction of resistance to oxacillin and could be used to confirm results for isolates of staphylococci from serious infections.
- The results of disk diffusion tests using a 30-µg cefoxitin disk and alternate breakpoints (see [Table 2C](#)) can be used to predict *mecA*-mediated oxacillin resistance in staphylococci. The cefoxitin disk test is equivalent to oxacillin MIC tests in sensitivity and specificity for *S. aureus*. For coagulase-negative staphylococci, the cefoxitin disk test has equivalent sensitivity to oxacillin MIC tests but greater specificity, i.e., the cefoxitin disk test more accurately identifies oxacillin-susceptible strains than the oxacillin MIC test.

#### 12.1.1.3 Testing Issues

- The addition of NaCl (2% w/v; 0.34 mol/L) is required for both agar and broth dilution testing of oxacillin.
- Prepare the inoculum using the direct colony suspension method (see [Section 8.2](#)) rather than the growth method (see [Section 8.3](#)).
- Incubate tests to detect MRS for a full 24 hours (rather than 16 to 20 hours) at  $35 \pm 2$  °C (testing at temperatures above 35 °C may not detect MRS) before reporting as susceptible. Resistance may be reported any time growth is observed after a minimum of 16 hours incubation.

#### 12.1.1.4 Reporting

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

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

Report MRSA and coagulase-negative staphylococci as resistant to all other penicillins, carbapenems, cepheems, and  $\beta$ -lactam/ $\beta$ -lactamase inhibitors, regardless of *in vitro* test results with those agents. This is

because most cases of documented MRS infections have responded poorly to  $\beta$ -lactam therapy, or because convincing clinical data have yet to be presented that document clinical efficacy for those agents for MRS infections.

For oxacillin-susceptible strains, report results for parenteral and oral cepheims,  $\beta$ -lactam/ $\beta$ -lactamase inhibitor combinations, and carbapenems, if tested, according to the results generated using routine interpretive criteria.

### 12.1.2 Oxacillin Screening Plates

The oxacillin salt-agar screening-plate procedure can be used in addition to the dilution methods described above for the detection of MRSA. Perform the test by inoculating a *S. aureus* isolate onto Mueller-Hinton agar that has been supplemented with NaCl (4% w/v; 0.68 mol/L) and that contains 6  $\mu$ g oxacillin/mL. Inoculate the agar from a direct colony suspension equivalent to a 0.5 McFarland standard using either a 1- $\mu$ L loop or a swab. Using a 1- $\mu$ L loop, spread the inoculum in an area 10 to 15 mm in diameter. Alternatively, using a swab, express excess fluid as for the disk diffusion test and then spot an area at least 10 to 15 mm in diameter or streak an entire quadrant. Incubate the plate at temperatures at  $35 \pm 2$  °C (testing at temperatures above 35 °C may not detect MRS) for 24 hours and examine carefully with transmitted light for evidence of small colonies (>1 colony) or a light film of growth, indicating oxacillin resistance (see Table 2C).<sup>9</sup> Do not reuse plates after incubation.

### 12.1.3 Vancomycin Resistance and Reduced Susceptibility to Vancomycin

#### 12.1.3.1 Vancomycin Resistance

From 2002 to 2005, a total of four *S. aureus* strains for which the vancomycin MICs ranged from 32 to 1024  $\mu$ g/mL were reported from the United States. All of these strains contained a *vanA* gene similar to that found in enterococci.<sup>10,11</sup> These strains are reliably detected by the broth microdilution reference method, the disk diffusion method and the vancomycin agar screen test (see below) when the tests are incubated for a full 24 hours at  $35 \pm 2$  °C.

#### 12.1.3.2 Reduced Susceptibility to Vancomycin

Strains of *S. aureus* and coagulase-negative staphylococci for which the vancomycin and teicoplanin MICs are in the intermediate range have been described.<sup>12,13</sup> The first occurrence of a strain of *S. aureus* with reduced susceptibility to vancomycin (MICs 4 to 16  $\mu$ g/mL) was reported from Japan in 1997,<sup>14</sup> followed by reports from the United States and France.<sup>15</sup> 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.

In order to recognize strains of staphylococci with reduced susceptibility to vancomycin (MICs 4 to 16  $\mu$ g/mL), MIC testing must be performed and the tests incubated for a full 24 hours at  $35 \pm 2$  °C. These strains 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 reduced susceptibility to vancomycin (see below); however, the performance of the vancomycin agar screen test has not been rigorously studied with strains for which the vancomycin MICs are 4  $\mu$ g/mL.<sup>16</sup> Until further data on the prevalence or clinical significance of these isolates are known, laboratories may choose to examine MRSA strains more carefully for elevated MICs to vancomycin.

### 12.1.4 Vancomycin Agar Screen

The vancomycin agar screening-plate procedure can be used for the detection of *S. aureus* with reduced susceptibility to vancomycin. Perform the test by inoculating an isolate of *S. aureus* onto Brain Heart Infusion (BHI) agar that has been supplemented with 6 µg/mL of vancomycin. Preferably, inoculate the agar from a direct colony suspension equivalent to a 0.5 McFarland standard using a micropipette to deliver a 10-µL drop to the agar surface. Alternatively, use a swab from which the liquid has been expressed as for the disk diffusion test. Spot an area at least 10 to 15 mm in diameter. Growth may be more difficult to detect using the swab method. Incubate the plate at 35 ± 2 °C in ambient air for a full 24 hours and examine carefully for evidence of small colonies (>1 colony) or a film of growth, suggesting reduced susceptibility to vancomycin (also see Table 2C). Organisms present in quantities greater than one colony should be re-identified and the MICs confirmed by a validated MIC method. Use of a susceptible quality control strain, such as *Enterococcus faecalis* ATCC® 29212, is critical to ensure specificity. *E. faecalis* ATCC® 51299 can be used as a positive control. Currently, there are insufficient data to recommend using this agar screen test for coagulase-negative staphylococci.

### 12.1.5 Clindamycin Resistance

Inducible clindamycin resistance can be identified using the method described in [Section 13](#).

## 12.2 Enterococci

### 12.2.1 Penicillin/Ampicillin Resistance

Enterococci may be resistant to penicillin and ampicillin because of production of low-affinity, penicillin-binding proteins (PBPs) or, rarely, because of the production of β-lactamase. Either the agar or broth dilution tests accurately detects isolates with altered PBPs, but will not reliably detect isolates that produce β-lactamase. The rare β-lactamase-producing strains of enterococci are detected best by using a direct, nitrocefin-based, β-lactamase test (see [Section 14.2](#)). A positive β-lactamase test predicts resistance to penicillin, and amino-, carboxy-, and ureidopenicillins. Certain enterococci may possess high-level resistance to penicillin (MICs ≥128 µg/mL) or ampicillin (MICs ≥64 µg/mL). Enterococci with lower 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, whereas strains with higher levels of penicillin (MICs ≥128 µg/mL) or ampicillin (MICs ≥64 µg/mL) resistance may not be susceptible to the synergistic effect.<sup>17,18</sup> Physicians' requests to determine the actual MIC of penicillin or ampicillin for blood and CSF isolates of enterococci should be considered.

### 12.2.2 Vancomycin Resistance

Accurate detection of vancomycin-resistant enterococci by the agar or broth dilution test requires incubation for a full 24 hours (rather than 16 to 20 hours) before reporting as susceptible and careful examination of the plates, tubes, or wells for evidence of faint growth. A vancomycin agar screen test may also be used, as described below and in [Table 2D](#).

### 12.2.3 Vancomycin Agar Screening Plates

The vancomycin agar screening-plate procedure can be used in addition to the dilution methods described above for the detection of vancomycin-resistant enterococci. Perform the test by inoculating an enterococcal isolate onto BHI agar that has been supplemented with 6 µg vancomycin/mL.<sup>19</sup> Inoculate the agar from a direct colony suspension equivalent to a 0.5 McFarland standard using either a 1- or 10-µL loop or a swab.<sup>20</sup> Using a loop, spread the inoculum in an area 10 to 15 mm in diameter.

Alternatively, use a swab, expressing as for the disk diffusion test and then spotting an area at least 10 to 15 mm in diameter. Incubate the plate at  $35 \pm 2$  °C in ambient air for a full 24 hours and examine carefully for evidence of growth, including small colonies (>1 colony) or a film of growth, indicating vancomycin resistance (also see [Table 2D](#)). Do not reuse plates after incubation.

#### 12.2.4 High-Level Aminoglycoside Resistance

High-level resistance to gentamicin and/or streptomycin indicates that an enterococcal isolate will not be killed by the synergistic action of a penicillin or glycopeptide combined with that aminoglycoside.<sup>17</sup> 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 [Table 2D](#)). Quality control of these tests is also explained in [Table 2D](#). Other aminoglycosides need not be tested, because their activities against enterococci are not superior to gentamicin or streptomycin.

### 12.3 Extended-Spectrum, β-Lactamase-Producing, Gram-Negative Bacilli

Extended-spectrum β-lactamases (ESBLs) are enzymes that arise by mutations in genes for common plasmid-encoded β-lactamases, such as TEM-1, SHV-1, and OXA-10, or may be only distantly related to a native enzyme, as in the case of the CTX-M β-lactamases. ESBLs may confer resistance to penicillins, cephalosporins, and aztreonam in clinical isolates of *Klebsiella pneumoniae*, *K. oxytoca*, *Escherichia coli*, *Proteus mirabilis*<sup>21</sup>, and other genera of the family Enterobacteriaceae.<sup>22</sup> Some of these strains will show MICs above those of the normal susceptible population but below the standard breakpoints for certain extended-spectrum cephalosporins or aztreonam; such strains may be screened for potential ESBL production by using the screening breakpoints listed in the table at the end of [Table 2A](#). Other strains may test intermediate or resistant by standard breakpoints to one or more of these agents. In all strains expressing ESBLs, the MICs for one or more of the extended-spectrum cephalosporins or aztreonam should decrease in the presence of clavulanic acid (see [ESBL table at the end of Table 2A](#)). Report all ESBL-producing strains as resistant to all penicillins, cephalosporins, and aztreonam. Susceptible or intermediate results for β-lactam/β-lactamase inhibitor combinations, cephamycins, oxacephems, and carbapenems should not be changed. For current recommendations regarding testing and reporting, see [Table 1](#) and the table at the end of [Table 2A](#) for ESBL screening and confirmatory tests.

## 13 Inducible Clindamycin Resistance

Macrolide-resistant isolates of *S. aureus*, coagulase-negative *Staphylococcus* spp., and beta-hemolytic streptococci may express constitutive or inducible resistance to clindamycin (methylation of the 23S rRNA encoded by the *erm* gene also referred to as MLS<sub>B</sub> [macrolide, lincosamide, and type B streptogramin] resistance) or may be resistant only to macrolides (efflux mechanism encoded by the *msrA* gene in staphylococci or a *mef* gene in streptococci).

Inducible clindamycin resistance can be detected using a disk approximation test.<sup>23,24</sup> This may be done on a standard blood agar plate used for the inoculum purity check or by using the standard disk diffusion procedure. For current recommendations regarding testing procedures, see [Tables 2C](#) (*Staphylococcus* spp.) and 2H (beta-hemolytic streptococci). Following incubation, report organisms that do not show flattening of the clindamycin zone adjacent to the erythromycin disk as “clindamycin susceptible.” Organisms that show flattening of the clindamycin zone adjacent to the erythromycin disk (referred to as a “D” zone) have inducible clindamycin resistance. Report such isolates as “clindamycin resistant.” A comment that “This isolate is presumed to be resistant based on detection of inducible clindamycin resistance. Clindamycin may still be effective in some patients.” may be included. Recommendations for quality control/quality assessment are given in [Table 3](#).

## 14 $\beta$ -Lactamase Tests

### 14.1 Purpose

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

A positive  $\beta$ -lactamase test result predicts the following:

- resistance to penicillin, ampicillin, and amoxicillin among *Haemophilus* spp., *N. gonorrhoeae*, and *M. catarrhalis*; and
- resistance to penicillin, and amino-, carboxy-, and ureidopenicillins among staphylococci and enterococci.

A negative  $\beta$ -lactamase test result does not rule out resistance due to other mechanisms. Do not use  $\beta$ -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  $\beta$ -lactams most often used for therapy.

### 14.2 Selecting a $\beta$ -Lactamase Test

Nitrocefin-based tests are the preferred method for testing *Haemophilus* spp., *N. gonorrhoeae*, *M. catarrhalis*, staphylococci, and enterococci.<sup>25</sup> Acidimetric  $\beta$ -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*.<sup>26</sup> Accurate detection of  $\beta$ -lactamase in staphylococci may require induction of the enzyme and incubation of a nitrocefin-based test for up to one hour. Induction can be easily accomplished by testing the growth from the zone margin surrounding an oxacillin or cefoxitin disk test. Care must be exercised when using these assays to ensure accurate results, including testing of known positive and negative control strains at the time clinical isolates are examined.

## 15 Reporting of MIC Results

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

## 15.1 Susceptible

The “susceptible” category implies that isolates are inhibited by the usually achievable concentrations of antimicrobial agent tested when the recommended dosage is used for the site of infection.

## 15.2 Intermediate

The “intermediate” category includes isolates with antimicrobial agent MICs that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates. The intermediate category implies clinical efficacy in body sites where the drugs are physiologically concentrated (e.g., quinolones and  $\beta$ -lactams in urine) or when a higher than normal dosage of a drug can be used (e.g.,  $\beta$ -lactams). The intermediate 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.

## 15.3 Resistant

The “resistant” category implies that isolates are not inhibited by the usually achievable systemic concentrations of the agent with normal dosage schedules, and/or they may have MICs that fall within the range where specific microbial resistance mechanisms are likely (e.g.,  $\beta$ -lactamases) and clinical efficacy has not been reliable in treatment studies.

# 16 Quality Control Procedures

## 16.1 Purpose

The goals of a quality control program are to assist in monitoring the following:

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

The goals are achieved by, but not limited to, the testing of quality control strains with known susceptibility to the antimicrobial agents to be tested.

## 16.2 Quality Control Responsibilities

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

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 FDA Quality System Regulation;
  - integrity of product; and
  - accountability and traceability to consignee.
- Laboratory (User):
    - storage under the environmental conditions recommended by the manufacturer (to prevent drug deterioration);
    - proficiency of personnel performing tests; and
    - adherence to the established procedure, e.g., inoculum preparation, incubation conditions, interpretation of end points.

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

### 16.3 Reference Strains for Quality Control

- Ideal reference strains for quality control of dilution tests have MICs that fall near the middle of the concentration range tested for all antimicrobial agents, e.g., an ideal control strain would be inhibited at the fourth dilution of a seven-dilution series, but strains with MICs at either the third or fifth dilution would also be acceptable. In certain instances with newer, more potent antimicrobial agents, it may be necessary to test additional quality control strains not normally tested in order to provide on-scale values.
- When three or fewer adjacent doubling dilutions of an antimicrobial agent are tested by dilution methods, quality control procedures must be modified. One possible alternative is to use one control organism with a modal MIC that is equal to, or no less than, one doubling dilution of the lower concentration and a second control organism with a modal MIC that is equal to, or no greater than, one doubling dilution of the higher concentration. The combination of results derived from testing these two strains should provide for at least one on-scale end point. For users of commercial systems, this strategy might be used best by selectively testing the most labile agents included in the panels, e.g., clavulanic acid combinations, methicillin, imipenem, and cefaclor.

The quality control strains used in the standard disk diffusion method have also been used often as control strains for dilution susceptibility tests. *S. aureus* ATCC<sup>®</sup> 25923, however, is of little value in dilution testing because of its extreme susceptibility to most drugs. *S. aureus* ATCC<sup>®</sup> 29213 is a weak,  $\beta$ -lactamase-producing strain that is a useful control strain for dilution tests. The *P. aeruginosa* ATCC<sup>®</sup> 27853 strain develops resistance to  $\beta$ -lactam antimicrobial agents after repeated transfers onto laboratory media, but this problem can be minimized by removing a new culture from storage at appropriate intervals (i.e., at least monthly), or whenever the strain begins to show resistance. While a perfect set of quality control strains may not exist, the strains listed below and in [Tables 3](#) and [3A](#) should fulfill the needs of most laboratories when performing standard reference dilution testing.

The following reference strains are recommended for controlling dilution tests:

- *Enterococcus faecalis* ATCC<sup>®</sup> 29212;
- *Enterococcus faecalis* ATCC<sup>®</sup> 51299;
- *Escherichia coli* ATCC<sup>®</sup> 25922;
- *Escherichia coli* ATCC<sup>®</sup> 35218;
- *Haemophilus influenzae* ATCC<sup>®</sup> 49247;
- *Haemophilus influenzae* ATCC<sup>®</sup> 49766;
- *Helicobacter pylori* ATCC<sup>®</sup> 43504;
- *Klebsiella pneumoniae* ATCC<sup>®</sup> 700603;
- *Neisseria gonorrhoeae* ATCC<sup>®</sup> 49226;
- *Pseudomonas aeruginosa* ATCC<sup>®</sup> 27853;
- *Staphylococcus aureus* ATCC<sup>®</sup> 29213;
- *Staphylococcus aureus* ATCC<sup>®</sup> 43300; and
- *Streptococcus pneumoniae* ATCC<sup>®</sup> 49619.

*Enterococcus faecalis* ATCC<sup>®</sup> 29212 and *Enterococcus faecalis* ATCC<sup>®</sup> 51299 are used as control organisms for high-level aminoglycoside and vancomycin screen tests (see [Table 2D](#)).

*Enterococcus faecalis* ATCC<sup>®</sup> 29212 (or alternatively *Enterococcus faecalis* ATCC<sup>®</sup> 33186) can be used to monitor Mueller-Hinton agar for unacceptable levels of thymidine when trimethoprim or sulfonamides are tested.

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

*Haemophilus influenzae* ATCC<sup>®</sup> 49247 is an ampicillin-resistant,  $\beta$ -lactamase-negative organism.

*Haemophilus influenzae* ATCC<sup>®</sup> 49766 is an ampicillin-susceptible organism that is more reproducible than *H. influenzae* ATCC<sup>®</sup> 49247 for controlling selected  $\beta$ -lactams.

*Klebsiella pneumoniae* ATCC<sup>®</sup> 700603 is used as a control for ESBL tests (see [Table 2A](#)). Proper maintenance is important for this strain; see the next section.

*Staphylococcus aureus* ATCC<sup>®</sup> 29213 and *Staphylococcus aureus* ATCC<sup>®</sup> 43300 are used as control organisms for oxacillin salt agar screening tests (see [Table 2C](#)).

#### 16.4 Storing and Testing Quality Control Strains

- Test the quality control strains by standard dilution test procedures described herein using the same materials and methods that are used to test clinical isolates.
- For prolonged storage, maintain stock cultures at  $\leq -20$  °C or below (preferably at  $\leq -60$  °C or below or in liquid nitrogen) in a suitable stabilizer (e.g., 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.
- Store working control cultures on tryptic soy agar (nonfastidious strains) or enriched chocolate agar (fastidious strains) slants at 2 to 8 °C and subculture each week for no more than three successive weeks. Prepare new working cultures at least monthly from frozen, freeze-dried, or commercial cultures.
- Before testing, subculture the strains onto agar plates to obtain isolated colonies. Subculture frozen or freeze-dried cultures twice prior to testing.
- Grow organisms and prepare log phase or direct colony suspensions for testing according to the recommended inoculum preparation procedures.
- A quality control culture can be used to monitor precision (repeatability) and accuracy of the dilution test as long as no significant change in the MICs not attributable to faulty methodology occurs. If an unexplained result suggests a change in the organism's inherent susceptibility, a fresh culture of the control strain should be obtained.
- Careful attention to organism maintenance (e.g., minimal subcultures) and storage (e.g., -60 °C or below) is especially important for quality control strains *E. coli* ATCC<sup>®</sup> 35218 and *K. pneumoniae* ATCC<sup>®</sup> 700603, because spontaneous loss of the plasmid encoding the  $\beta$ -lactamase has been documented. Plasmid loss leads to quality control results outside the acceptable limit, such as decreased MICs for *E. coli* ATCC<sup>®</sup> 35218 with enzyme labile penicillins (e.g., ampicillin, piperacillin, and ticarcillin) and decreased MICs for *K. pneumoniae* ATCC<sup>®</sup> 700603 with cephalosporins and aztreonam.

#### 16.5 Batch or Lot Quality Control

- (1) Test each new batch or lot of macrodilution tubes, microdilution trays, or agar dilution plates with the appropriate reference strains to determine if MICs obtained with the batch fall within the expected range (see [Tables 3](#) and [3A](#)); if they do not, the batch must be rejected.
- (2) Incubate at least one uninoculated tube, microdilution tray, or agar plate from each batch overnight to verify sterility of the medium.
- (3) New lots of Mueller-Hinton broth used to prepare macrodilution tubes or microdilution trays may need to be tested for acceptable cation content. For Mueller-Hinton broth, determine the MIC of gentamicin for *P. aeruginosa* ATCC<sup>®</sup> 27853 and compare it with the expected range (see [Table 3](#)). If

the MIC is low, the broth may need to be supplemented with cations as directed in [Section 10.1](#). When daptomycin is tested, levels of calcium cations in broth should be 50 mg/L. Mueller-Hinton agar may also be tested for appropriate cation content by disk diffusion with *P. aeruginosa* ATCC® 27853, using a 10- $\mu$ g gentamicin disk, and with *Staphylococcus aureus* ATCC® 25923, using a 30- $\mu$ g daptomycin disk as described in CLSI document [M2—Performance Standards for Antimicrobial Disk Susceptibility Tests](#). To be acceptable, the inhibition zone diameter must fall within the specified gentamicin or daptomycin control range as defined in the most current version of the [M100](#) supplement to the [M2](#) document.

- (4) Records should be kept of the lot numbers of all materials and reagents used in performing susceptibility tests.
- (5) *Haemophilus influenzae* ATCC® 10211 is recommended as a useful additional quality control strain to verify the growth promotion properties of HTM. In particular, manufacturers of HTM are encouraged to use *H. influenzae* ATCC® 10211 as a quality control test strain.

## 16.6 MIC Quality Control Limits

Acceptable MIC quality control limits for a single quality control test (single-drug/single-organism combination) are listed in [Tables 3](#) and [3A](#). The overall performance of the test system should be monitored using these limits by testing the appropriate control strains each day the test is performed or, if satisfactory performance is documented (see [Section 16.7.2.1](#)), test the control strains weekly (see [below](#)).

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

The weekly quality control testing option outlined below is applicable to routine MIC tests only. Quality control testing should be performed each test day for MIC tests performed less than once a week.

### 16.7.1 Daily Testing

Performance is satisfactory for daily QC testing when no more than 3 out of 30 consecutive results for each antimicrobial agent/organism combination are outside the acceptable limit stated in [Tables 3](#) and [3A](#). Corrective action by the laboratory is required when this frequency is exceeded.

### 16.7.2 Weekly Testing

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

- Test all applicable control strains for 20 or 30 consecutive test days and document results.
- To convert from daily to weekly quality control testing, no more than 1 out of 20 or 3 out of 30 MICs for each antimicrobial agent/organism combination may be outside the acceptable MIC limits stated in [Tables 3](#) and [3A](#).

#### 16.7.2.2 Implementing Weekly Quality Control Testing

- Weekly quality control testing may be performed once satisfactory performance has been documented (see [Section 16.7.2.1](#)).
- ◆ Perform quality control testing once per week and whenever any reagent component of the test (e.g., a new lot of broth from the same manufacturer) is changed.

- ◆ If any of the weekly quality control results are out of the acceptable range, corrective action is required (see [Section 16.9](#)).
- If a new antimicrobial agent is added or broth or agar from a different manufacturer is used, it must be tested for 20 or 30 consecutive days and satisfactory performance documented before it can be tested on a weekly schedule. In addition, 20 or 30 days of testing is required if a major change in the method of reading test results is implemented, such as conversion from a visual reading of MICs to an instrument reading or conversion in the type of panel used (i.e., changing from breakpoint to MIC panels).
- These guidelines can also be used for testing systems in which an MIC is determined using three or fewer adjacent doubling dilutions of an antimicrobial agent.
- For some drugs, quality control records may indicate the need for testing to be done more frequently than once a week because of the relatively rapid degradation of the drug (see [Sections 9.2.1](#) and [10.2.2](#) and [Table 3C](#)).

## 16.8 Frequency of Quality Control Testing for Screening Tests

The frequency of quality control screening tests is as follows:

- Quality control of vancomycin and high-level aminoglycoside screen plates may be performed weekly if these tests are routinely used (i.e., at least once a week) in the laboratory, and criteria for converting from daily to weekly testing has been met.
- Quality control of screening tests may be needed each day of testing if the test is not performed routinely (at least once a week) or if the antimicrobial agent is labile (e.g., oxacillin agar screen for *S. aureus*).

## 16.9 Corrective Action

### 16.9.1 Out-of-Control Result Due to an Obvious Error

If out-of-control results occur for obvious reasons, document the reason and retest the strain on the day that the error is observed. If the repeated result is within range, no further corrective action is required. Obvious reasons for the out-of-control results include:

- use of the wrong control strain;
- obvious contamination of the strain or the medium;
- inadequate volume of broth in tubes or wells; or
- inadvertent use of the wrong incubation temperature or conditions.

### 16.9.2 Out-of-Control Result Not Due to an Obvious Error

#### 16.9.2.1 Immediate Corrective Action

If no obvious reason for the out-of-control result is apparent, immediate corrective action is required.

- Test the out-of-control antimicrobial agent/organism combination on the day the error is observed and monitor for a total of five consecutive test days. Document all results.
- If all five MICs for the antimicrobial agent/organism combination are within the acceptable ranges, as defined in [Tables 3](#) and [3A](#), no additional corrective action is necessary.
- If any of the five MICs is still outside the acceptable range, additional corrective action is required (see [Section 16.9.2.2](#)).
- Daily control tests must be continued until final resolution of the problem is achieved.

#### 16.9.2.2 Additional Corrective Action

When immediate corrective action does not resolve the problem, the problem is likely due to a system rather than a random error. The following common sources of error should be investigated to verify that:

- the turbidity standard has not expired, is stored properly, meets performance requirements (see [Section 8.1](#)), and was adequately mixed prior to use;
- all materials used were within their expiration dates and stored at the proper temperature;
- the incubator was at proper temperature and atmosphere;
- other equipment used (e.g., pipettors) was functioning properly;
- plates were stored at proper temperature;
- the control strain has not changed and was not contaminated;
- inoculum suspensions were prepared and adjusted correctly; and
- inoculum for the test was prepared from a plate incubated for the correct length of time and in no case more than 24 hours old.

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

Once the problem is corrected, documentation of satisfactory performance for another 20 or 30 consecutive days is required in order to return to weekly quality control testing (see [Section 16.7.2.1](#)).

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

Whenever an out-of-control result occurs or corrective action is necessary, careful assessment of whether to report patient test results should be made on an individual patient basis, taking into account if the source of the error, when known, is likely to have affected relevant patient test results. Options to consider include suppressing the results for an individual antimicrobial agent; retrospectively reviewing individual patient or cumulative data for unusual patterns; and using an alternate test method or a reference laboratory until the problem is resolved.

## 16.11 Verification of Patient Test Results

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

- the antimicrobial susceptibility results are consistent with the identification of the isolate;
- the results from individual agents within a specific drug class follow established hierarchy of activity rules (e.g., third-generation cephalosporins are more active than first- or second-generation cephalosporins against Enterobacteriaceae); and
- the isolate is susceptible to those agents for which resistance has not been documented (e.g., vancomycin and *Streptococcus* spp.) and for which only “susceptible” interpretive criteria exist in [M100](#).

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

- inaccurate determination of MIC endpoint;
- transcription errors;
- contamination of the test (e.g., recheck purity plates);
- use of a defective panel, plate, or card (e.g., broken tray or underfilled well); and
- previous results on the patient (e.g., Did the patient have the same isolate with an unusual antibiogram previously?).

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 [Table 8 of M100](#). Each laboratory must develop its own policy for verification of unusual or inconsistent antimicrobial susceptibility test results. This policy should emphasize those results that may significantly impact patient care.

## 16.12 Other Control Procedures

### 16.12.1 Growth Control

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

### 16.12.2 Purity Control

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

### 16.12.3 Inoculum Control

Periodically perform plate counts with representative inocula to ensure that the 0.5 McFarland standard and the procedures for standardizing and diluting inocula remain under control. To do this, remove samples for plate counts immediately after inoculation from the growth-control well of microdilution trays, the growth-control tube of a macrodilution series, or in agar dilution a random reservoir well of the replicator seed block (see [Section 10.3.1.3](#)).

### 16.12.4 End Point Interpretation Control

Monitor end point interpretation periodically to minimize variation in the interpretation of MIC end points among observers. All laboratory personnel who perform these tests should independently read a selected set of dilution tests. Record the results and compare to the results obtained by an experienced reader. All readers should agree within  $\pm 1$  twofold concentration increment of one another.<sup>27</sup>

## 17 Limitations of Dilution Test Methods

### 17.1 Application to Various Organism Groups

The dilution methods described in this document are standardized for testing rapidly growing pathogens, which include *Staphylococcus* spp., *Enterococcus* spp., the Enterobacteriaceae, *P. aeruginosa*, *Pseudomonas* spp., *Acinetobacter* spp., *Burkholderia cepacia*, *Stenotrophomonas maltophilia*, and potential agents of bioterrorism, including *Bacillus anthracis*, *Burkholderia mallei*, *B. pseudomallei*, *Francisella tularensis*, and *Yersinia pestis* and they have been modified for testing some fastidious organisms, such as *Haemophilus* spp. ([Table 2E](#)), *N. gonorrhoeae* ([Table 2F](#)), *N. meningitidis* ([Table 2L](#)), streptococci ([Tables 2G](#) and [2H](#)), and *Helicobacter pylori* ([Table 2J](#)). For additional guidance on standardized susceptibility testing of organisms not presently included in M2 or M7, please refer to the most current edition of CLSI document [M45—Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria](#).

### 17.2 Misleading Results

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

- first- and second-generation cephalosporins and aminoglycosides against *Salmonella* and *Shigella* spp.;
- all  $\beta$ -lactam antimicrobial agents (except oxacillin, methicillin, and nafcillin) against methicillin-resistant staphylococci;
- cephalosporins, aminoglycosides (except testing for high-level resistance), clindamycin, and trimethoprim-sulfamethoxazole against enterococci;
- cephalosporins against *Listeria* spp.; and
- cephalosporins and aztreonam against ESBL-producing *K. pneumoniae*, *K. oxytoca*, *E. coli*, and *P. mirabilis*.

### 17.3 Emergence of Resistance

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

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

## 18 Screening Tests

Screening tests for oxacillin-resistant *Staphylococcus aureus* and high-level aminoglycoside-resistant enterococci have been shown to be comparable in reliability to standard methods for detecting clinically significant resistance; additional confirmatory tests are unnecessary. Limitations of other screening tests, e.g., for vancomycin resistance in enterococci (Table 2D) and *S. aureus* (Table 2C) and ESBLs in certain Enterobacteriaceae (Table 2A) and the necessity for further confirmation are given in the individual tables.

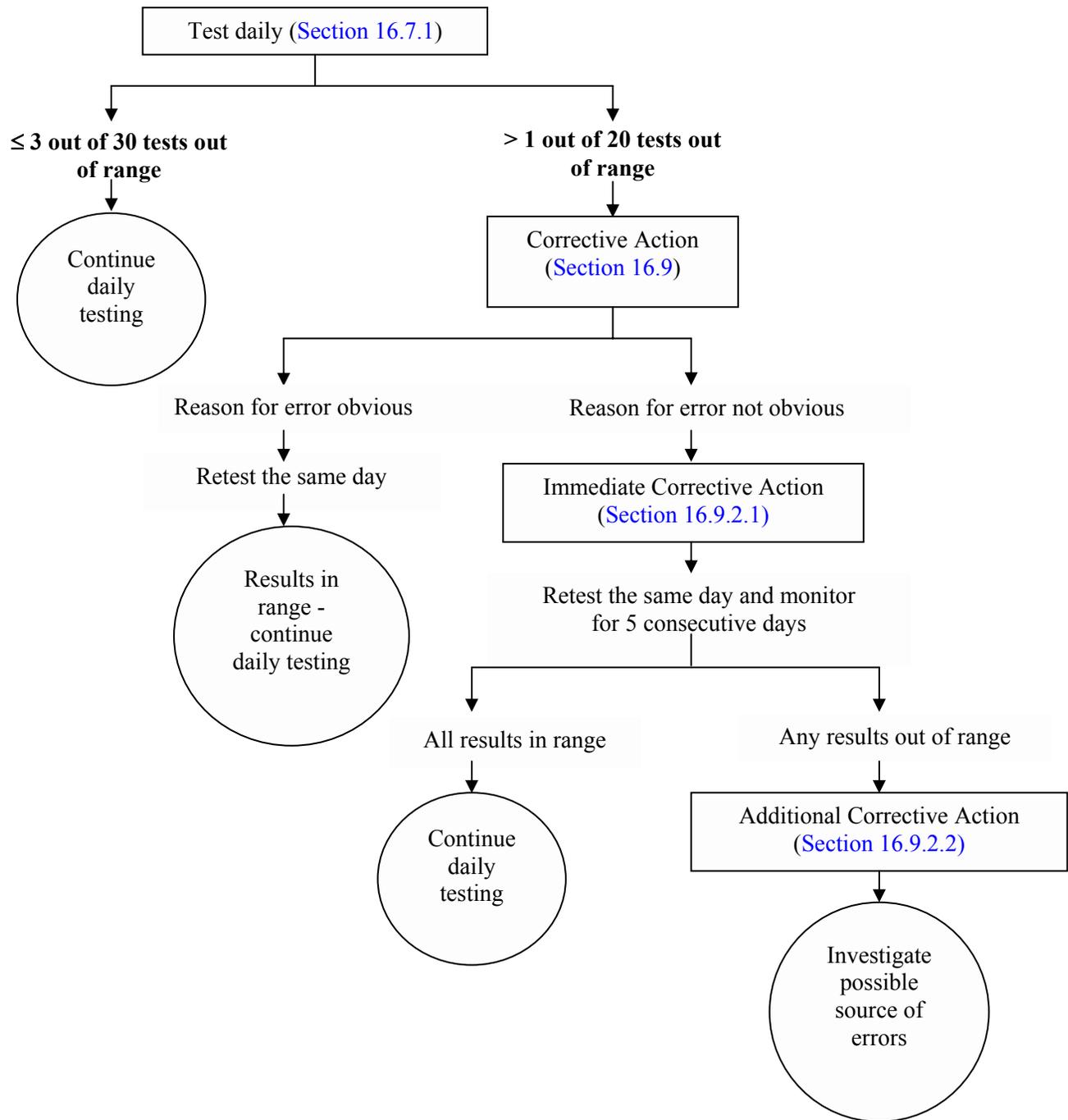
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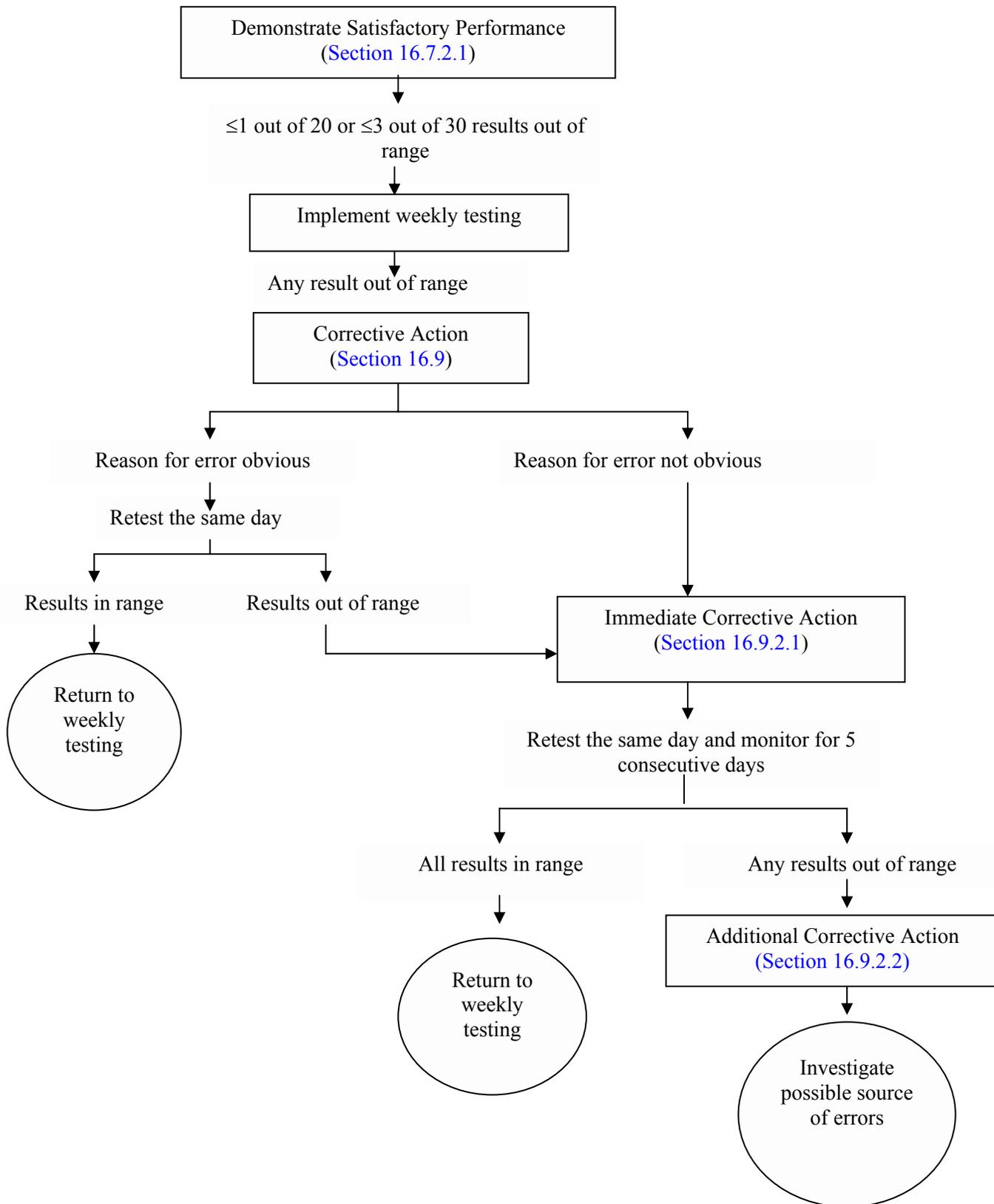
**Appendix A. Quality Control Protocol Flow Charts**

**Aerobic Dilution Daily Quality Control Testing Protocol**



**Appendix A. (Continued)**

**Aerobic Dilution Weekly Quality Control Testing Protocol**



**Clinical and Laboratory Standards Institute consensus procedures include an appeals process that is described in detail in Section 8 of the Administrative Procedures. For further information, contact CLSI or visit our website at [www.clsi.org](http://www.clsi.org).**

## Summary of Comments and Subcommittee Responses

M7-A6: *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Sixth Edition*

### General

1. What is the intention of the “Warning” (page 23 of the M100-S14 Vol. 24, No. 1 standards for antimicrobial susceptibility testing) regarding not routinely reporting clindamycin, macrolides, etc. for bacteria isolated from the “CSF”? Should this be interpreted in its narrowest sense, or does CLSI consider all central nervous system (CNS) sites similarly to CSF? Specifically, would it be incorrect to report clindamycin, etc. for a bacterial brain abscess aspirate? Infectious disease textbooks consider clindamycin as an acceptable alternative to treat brain abscesses.
  - **The WARNING box in M100 refers only to bacterial meningitis. Clindamycin is mentioned among alternative therapies useful for brain abscess (*Principles and Practice of Infectious Diseases*, G. L. Mandell, J. E. Bennett, and R. Dolin (eds.), 5<sup>th</sup> edition, Philadelphia, Churchill Livingstone, Inc., 2000), so it would not be incorrect to report clindamycin susceptibility test results on an aspirate from a brain abscess. There are insufficient data to provide comprehensive guidance on which test results to report routinely for pathogens isolated from brain abscesses.**

### Table 2A

2. Could you clarify the comment in M100 regarding the Warning for *Salmonella* and *Shigella* in Table 2A, comment (5) on page 35? The comment states that first- and second-generation cephalosporins should not be reported as susceptible. Does that comment include the cephamycins also?
  - **Yes, the statement has been clarified.**

### Table 2B

3. On page 95, footnote j states that “Other non-Enterobacteriaceae include *Pseudomonas* spp. and other nonfastidious, glucose-nonfermenting, gram-negative bacilli except for *Acinetobacter* spp., *Burkholderia cepacia*, and *Stenotrophomonas maltophilia*.” On page 108 under general comment (1), it states “Non-Enterobacteriaceae include *Acinetobacter* spp., *S. maltophilia*, *Pseudomonas* spp., and other...” Is this not contradicting the statement on page 95?
  - **In order to avoid this confusion in the future, the wording in Table 1, footnote j has been revised.**

### Table 2C

4. There appears to be a discrepancy between the disk diffusion and MIC sections of the current M100 document as related to oxacillin:

For disk diffusion testing of non-*S. epidermidis* coagulase negative staphylococci, disk diffusion “R” isolates that are *mecA*/PBP2a negative should be reported as “S.” (Pg. 42)

However, for MIC testing of these same non-*S. epidermidis* coagulase negative staphylococci, isolates that are *mecA*/PBP 2a negative should be reported as “S” if oxacillin MICs are between 0.5 to 2 mcg/mL, but as “R” if MICs are > 4 mcg/mL. (Pg. 105)

If moderately to highly oxacillin resistant *mecA*/PBP2a negative isolates should be reported as “R” when performing the MIC test, shouldn’t this also be the case when doing disk diffusion? Put another way, shouldn’t the disk diffusion criteria also have a zone size below which a report of “R” be made regardless of *mecA*/PBP 2a findings?

- **The reason for reporting strains exhibiting MICs  $\geq 4$   $\mu\text{g/mL}$  as oxacillin resistant despite *mecA*/PBP2a status is based on pharmacokinetic/pharmacodynamic data and the possibility that other resistance mechanisms not yet discovered may be responsible for the increased MICs. Unfortunately, there is no zone diameter or range of zone diameters that correlates exactly with MICs  $\geq 4$   $\mu\text{g/mL}$ . The data from the CLSI study used to establish the revised interpretive criteria (Tenover et al, *J Clin Microbiol.* 37: 4051-4058) show that 108 of 110 (98.2%) isolates with zone diameters of 6 mm (i.e., no zone) were *mecA*-positive. Of 42 strains with zone diameters in the 7 to 17 mm range, 15 (35.7%) were *mecA*-positive and 26 (64.3%) were *mecA* negative. Therefore, based on these data, it would be possible to do the following: if there is no zone to oxacillin, report as resistant; if there is any zone  $\geq 7$  mm, then perform a *mecA* test or a cefoxitin disk test for a definitive answer. However, recent studies show that using the cefoxitin disk in place of the oxacillin disk gives better correlation with *mecA* status for coagulase-negative staphylococci, and the cefoxitin zone is much easier to read.**
5. I am currently revising my SOP for  $\beta$ -lactamase testing of staphylococci, and I am training my staff to follow the CLSI/NCCLS guidelines stated in Table 2C of document M100-S14. Today one of my techs has a patient with a penicillin MIC of  $<0.03$   $\mu\text{g/mL}$ . Per CLSI/NCCLS, we should simply report the penicillin as susceptible. My tech did both a direct and an induced  $\beta$ -lactamase, and they were both positive. We are having a difficult time understanding why CLSI/NCCLS wants us to call the penicillin susceptible when we are getting a positive  $\beta$ -lactamase result.

Following CLSI/NCCLS and reporting the penicillin susceptible on this patient is making me uncomfortable, because I know that it is  $\beta$ -lactamase positive. My tech is asking me to explain why CLSI/NCCLS is telling us not to do the  $\beta$ -lactamase testing when the MIC is  $<0.03$   $\mu\text{g/mL}$ , and I am hoping that you can help me provide her an answer. After this patient, if we follow the CLSI/NCCLS guidelines, and stop doing the  $\beta$ -lactamase testing on patients with an MIC of  $<0.03$ , we will always feel uncomfortable knowing that it could be  $\beta$ -lactamase positive. Any information you can provide that will help us understand this will be greatly appreciated.

- **The CLSI/NCCLS recommendation is not what is stated above. Comment 6 in Table 2C of M7-M100-S14 (now comment 8 in M7-M100-S15) states: “A penicillin MIC of  $\leq 0.03$   $\mu\text{g/mL}$  usually implies lack of  $\beta$ -lactamase production, and MICs of  $\geq 0.25$   $\mu\text{g/mL}$  should be considered resistant; staphylococci with penicillin MICs between 0.06 to 0.12  $\mu\text{g/mL}$  may or may not produce  $\beta$ -lactamase, and an induced  $\beta$ -lactamase test can clarify these MICs (see M7-A6, Section 10.2).” Using CLSI reference methods, it should be extremely rare to find strains of staphylococci that exhibit penicillin MICs  $\leq 0.03$   $\mu\text{g/mL}$  and that produce  $\beta$ -lactamase; however, should a strain be determined to produce  $\beta$ -lactamase, it should be reported as penicillin resistant despite the penicillin MIC. Those laboratories using commercial systems should follow the manufacturer’s recommendations for guidance in this situation.**

#### Table 2E

6. I am aware that the antibiotic tested is the one to be reported; however, I need clarification on how to address the reporting of doxycycline with the fastidious organisms when the tetracycline interpretation is resistant or intermediate. In Tables 2A, 2B, 2C, and 2D of M100-S15, all tetracycline comments end by stating, “...However, some organisms that are intermediate or resistant to tetracycline may be susceptible to doxycycline...” But in Tables 2E (*Haemophilus*) and 2G (*S. pneumoniae*), this statement is not part of the comment. Am I to assume this statement does not hold true for the fastidious organisms or can I deduce it does? Literature leads me to believe it does. Should tetracycline R or I be equated with doxycycline resistance? Will CLSI be developing zone sizes for doxycycline in the future?

Some physicians are hesitant to use doxycycline because of presumed inactivity. Clinicians assume isolates reported resistant to tetracycline are also doxycycline resistant. Our pharmacologist notes this assumption does not agree with the literature and that extrapolating our tetracycline susceptibility data to doxycycline has no direct application. It doesn’t relate to therapy or help in clinical studies. Citing literature, she says doxycycline

is consistently active against all common typical and atypical bacterial causes of pneumonia. She is concerned this assumption of tetracycline resistance (which is common for *S. pneumoniae*) implying doxycycline resistance as well is sending those not familiar with infectious disease in the wrong direction (i.e., towards the use of more expensive alternatives with more harmful side effects).

- **Tetracycline-susceptible isolates of various species are susceptible to doxycycline and minocycline. However, some organisms that are intermediate or resistant to tetracycline may be susceptible to doxycycline or minocycline. Currently, there are no interpretive criteria for doxycycline and minocycline against pneumococci and *H. influenzae*. Until additional studies are undertaken or reviewed by CLSI, it is premature to use tetracycline to predict doxycycline or minocycline resistance.**

Table 2G

7. Reading the CLSI documents of 2005, I wonder why the D-test that looks for inducible clindamycin resistance is not described for *Streptococcus pneumoniae*. Can you give me the reason behind this?
- **Isolates of *Streptococcus pneumoniae* can have *erm*-mediated resistance to erythromycin. However, the vast majority of these isolates are also resistant to clindamycin (i.e., the constitutive resistance phenotype). Rare isolates of pneumococci may have inducible resistance; however, the clinical significance of this has not been established. Therefore, routine testing for inducible clindamycin resistance is not recommended for this species.**

## Summary of Delegate Comments and Subcommittee Responses

*M7-A7: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Seventh Edition*

### Section 9.2.1, Procedure

1. Number (2) - Other CLSI standards include how many mL of agar to fill the plate with in addition to the maximum depth.
  - **The subcommittee will review this issue and address this in the next edition of the document.**
2. Number (4) - Add final pH of agar like in Section 9.1.1 (5).
  - **Text has been added to refer the reader to Section 9.1.1 (3) for final pH.**

### Section 10.1, Mueller-Hinton Broth

3. Number (3) - Do you need to check the pH of the Mueller-Hinton after addition of cations like in Section 10.1 (2)?
  - **In the experience of some, checking the pH before or after the addition of cations to Mueller-Hinton broth does not seem to make a difference, although it would be preferable to check the pH after the addition of all the components.**

### Section 11.2.1, Agar Medium

4. Do you need to include the pH of the GC agar?
  - **The exact pH range that can be used has not been determined. It is our understanding that the major laboratories doing GC agar dilution have not made it a practice to require a certain pH and do not know what the allowable range might be. There are ongoing studies that should provide the proper pH range that will be included in the next edition of the document.**

### Section 12.2.3, Vancomycin Agar Screening Plates

5. Include the statement, “Do not reuse plates after incubation” like in Section 12.1.2.
  - **This text has been added as suggested.**

**NOTES**

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**NOTES**

## The Quality System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management system approach in the development of standards and guidelines, which facilitates project management; defines a document structure via a template; and provides a process to identify needed documents. The approach is based on the model presented in the most current edition of CLSI/NCCLS document [HS1](#)—*A Quality Management System Model for Health Care*. The quality management system approach applies a core set of “quality system essentials” (QSEs), basic to any organization, to all operations in any healthcare service’s path of workflow (i.e., 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 quality system essentials (QSEs) are:

Documents & Records Organization Personnel	Equipment Purchasing & Inventory Process Control	Information Management Occurrence Management Assessment	Process Improvement Service & Satisfaction Facilities & Safety
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M7-A7 addresses the quality system essentials (QSEs) indicated by an “X.” For a description of the other documents listed in the grid, please refer to the Related CLSI/NCCLS Publications section on the following page.

Documents & Records	Organization	Personnel	Equipment	Purchasing & Inventory	Process Control	Information Management	Occurrence Management	Assessment	Process Improvement	Service & Satisfaction	Facilities & Safety
X GP2					X M2 M6 M11 M23 M29 M39 M45						M29

Adapted from CLSI/NCCLS document [HS1](#)—*A Quality Management System Model for Health Care*.

### Path of Workflow

A path of workflow is the description of the necessary steps to deliver the particular product or service that the organization or entity provides. For example, CLSI/NCCLS document [GP26](#)—*Application of a Quality Management System Model for Laboratory Services* defines a clinical laboratory path of workflow which consists of three sequential processes: preexamination, examination, and postexamination. All clinical laboratories follow these processes to deliver the laboratory’s services, namely quality laboratory information.

M7-A7 addresses the clinical laboratory path of workflow steps indicated by an “X.” For a description of the other documents listed in the grid, please refer to the Related CLSI/NCCLS Publications 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 M2 M11 M45	X M2 M11 M45	X M2 M11 M45	X M2 M11 M45	

Adapted from CLSI/NCCLS document [HS1](#)—*A Quality Management System Model for Health Care*.

## Related CLSI/NCCLS Publications\*

- GP2-A4**      **Clinical Laboratory Technical Procedure Manuals; Approved Guideline—Fourth Edition (2002).** This document provides guidance on development, review, approval, management, and use of policy, process, and procedure documents in the laboratory testing community.
- M2-A9**      **Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Ninth Edition (2006).** This document describes current recommended methods for disk susceptibility testing, criteria for quality control testing, and updated tables for interpretive zone diameters.
- M6-A2**      **Protocols for Evaluating Dehydrated Mueller-Hinton Agar; Approved Standard—Second Edition (2005).** This document includes procedures for evaluating production lots of dehydrated Mueller-Hinton agar, and for the development and application of reference media.
- M11-A6**      **Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard—Sixth Edition (2003).** This document provides reference methods for the determination of minimal inhibitory concentrations (MICs) of anaerobic bacteria by broth macrodilution, broth microdilution, and agar dilution. Interpretive and quality control tables are included.
- M23-A2**      **Development of *In Vitro* Susceptibility Testing Criteria and Quality Control Parameters; Approved Guideline—Second Edition (2001).** This document addresses the required and recommended data needed for the selection of appropriate interpretive standards and quality control guidelines for new antimicrobial agents.
- M29-A3**      **Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Third Edition (2005).** Based on U.S. regulations, this document provides guidance on the risk of transmission of infectious agents by aerosols, droplets, blood, and body substances in a laboratory setting; specific precautions for preventing the laboratory transmission of microbial infection from laboratory instruments and materials; and recommendations for the management of exposure to infectious agents.
- M39-A2**      **Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data; Approved Guideline—Second Edition (2005).** This document describes methods for the recording and analysis of antimicrobial susceptibility test data, consisting of cumulative and ongoing summaries of susceptibility patterns of clinically significant microorganisms.
- M45-P**      **Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Proposed Guideline (2005).** 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 M2, M7, or M11. The tabular information in this document presents the most current information for drug selection, interpretation, and quality control for the infrequently isolated or fastidious bacterial pathogens included in this guideline.
- M100-S16**      **Performance Standards for Antimicrobial Susceptibility Testing; Sixteenth Informational Supplement (2006).** This document provides updated tables for the antimicrobial susceptibility testing standards for disk (M2-A9) and MIC (M7-A7).

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

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