CLINICAL AND LABORATORY STANDARDS INSTITUTE®

28th Edition

M100

Performance Standards for Antimicrobial Susceptibility Testing

This document includes updated tables for the Clinical and Laboratory Standards Institute antimicrobial susceptibility testing standards M02, M07, and M11.

A CLSI supplement for global application.

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Performance Standards for Antimicrobial Susceptibility Testing

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Abstract

The data in the tables are valid only if the methodologies in CLSI documents M02,[1] M07,[2] and M11[3] are followed. These standards contain information about broth disk (M02[1]) and dilution (M07[2] and M11[3]) test procedures for aerobic and anaerobic bacteria, respectively.

Clinicians depend heavily on information from the microbiology laboratory for treating their seriously ill patients. The clinical importance of antimicrobial susceptibility test results demands that these tests be performed under optimal conditions and that laboratories have the capability to provide results for the newest antimicrobial agents.

The tables presented in M100 represent the most current information for drug selection, interpretation, and quality control using the procedures standardized in M02,[1] M07,[2] and M11.[3] Users should replace previously published tables with these new tables. Changes in the tables since the previous edition appear in boldface type.

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Overview of Changes

This supplement replaces the previous edition of the supplement, M100, 27th ed., published in 2017. This list includes the major changes in this document. Other minor or editorial changes were made to the general formatting and to some of the table footnotes and comments. Changes to the tables since the previous edition appear in boldface type. The following are additions or changes unless otherwise noted as a **"*deletion*."**

- **General:**
  - Revised nomenclature:
    - *Propionibacterium acnes* to *Cutibacterium* (formerly *Propionibacterium*) *acnes*
    - *Clostridium difficile* to *Clostridioides* (formerly *Clostridium*) *difficile*
    - *Enterobacter aerogenes* to *Klebsiella* (formerly *Enterobacter*) *aerogenes*
    - *Fusobacterium nucleatum* to *Fusobacterium* spp.
    - β-lactam/β-lactamase inhibitor combinations to β-lactam combination agents
    - Folate pathway inhibitor to folate pathway antagonist
    - Methicillin-resistant *Staphylococcus aureus* (MRSA) salt agar to oxacillin salt agar
    - To align with the International Organization for Standardization, changed the name of the inoculum preparation method in all appropriate tables from growth method to broth culture method and changed direct colony suspension to colony suspension

- **CLSI Breakpoint Additions/Revisions Since 2010:**
  - Alphabetized antimicrobial agent listings

  - Added:
    - Ceftazidime-avibactam breakpoints for *Enterobacteriaceae* (p. xxix) and *Pseudomonas aeruginosa* (p. xxx)
    - Ceftolozane-tazobactam disk diffusion breakpoints for *Enterobacteriaceae* (p. xxix)
    - Dalbavancin breakpoints for *Staphylococcus* spp. (p. xxx), *Enterococcus* spp., (vancomycin susceptible) (p. xxx), *Streptococcus* spp. β-hemolytic group (p. xxxi), and *Streptococcus* spp. viridans group (p. xxxi)

- **CLSI Epidemiological Cutoff Value Additions/Revisions Since 2015:**
  - Revised:
    - Piperacillin-tazobactam susceptible and intermediate breakpoint for anaerobes (p. xxxii)

- **CLSI Archived Resources:**
  - Added new table with Web addresses for tables on CLSI website containing archived breakpoints and test methods (p. xxxii)

Overview of Changes (Continued)

- **Instructions for Use of Tables:**
  - Added antimicrobial stewardship team to decision-making group for selecting appropriate antimicrobial agents to test and report and to the instructions for generating cumulative antibiograms (p. 1)
  - Revised example of therapy-related comment for use in a patient report (p. 7)
  - In **Warning** section, revised the table to clarify the drugs of choice for treating bacteria isolated from CSF (p. 8)
  - In **Routine, Supplemental, Screening, Surrogate Agent, and Equivalent Agent Testing to Determine Susceptibility and Resistance to Antimicrobial Agents** section:
    - **Supplemental Tests – Optional** table (p. 11):
      - Added EDTA-modified carbapenem inactivation method (eCIM) information
      - Added *P. aeruginosa* to organisms for modified carbapenem inactivation method (mCIM)
      - ***Deleted*** modified Hodge test
  - In **Surrogate Agent Tests** table (p. 12):
    - Added *Staphylococcus schleiferi* to list of species that cannot be reliably tested with cefoxitin
    - Added note to the results information for testing cefoxitin as a surrogate agent for oxacillin with *Staphylococcus epidermidis*
    - Clarified the cefazolin results comment
  - In **Examples of Equivalent Agent Tests** table (p. 13):
    - Added ampicillin as a surrogate to predict the results of amoxicillin for anaerobes
- **Table 1A. Suggested Groupings of Antimicrobial Agents Approved by the US Food and Drug Administration for Clinical Use That Should Be Considered for Testing and Reporting on Nonfastidious Organisms by Microbiology Laboratories in the United States:**
  - Added:
    - Ceftazidime-avibactam in test/report group B for *Enterobacteriaceae* and *P. aeruginosa* (p. 16)
    - Footnote regarding minimal inhibitory concentration (MIC) testing only for *S. aureus* and most coagulase-negative staphylococci (CoNS) for *Staphylococcus* spp. and oxacillin (p. 16)
    - Dalbavancin in test/report group C for *Staphylococcus* spp. and *Enterococcus* spp. (p. 17)

Overview of Changes (Continued)

- Clarified:
  - Recommendations for detecting oxacillin resistance (p. 20)
  - Recommendations on *Enterococcus* with low-level penicillin or ampicillin resistance when combination therapy with a β-lactam is being considered (p. 21)

- Revised recommendations for staphylococci that test susceptible to gentamicin to only use gentamicin in combination with other active agents that also test susceptible (p. 20)

- **Table 1B. Suggested Groupings of Antimicrobial Agents Approved by the US Food and Drug Administration for Clinical Use That Should Be Considered for Testing and Reporting on Fastidious Organisms by Microbiology Laboratories in the United States:**
  - Added:
    - Dalbavancin in test/report group C for *Streptococcus* β-hemolytic group and *Streptococcus* spp. viridans group (p. 23)
    - Footnote specifying the approved organisms for testing dalbavancin (p. 23)

- **Table 1C. Suggested Groupings of Antimicrobial Agents Approved by the US Food and Drug Administration for Clinical Use That Should Be Considered for Testing and Reporting on Anaerobic Organisms by Microbiology Laboratories in the United States:**
  - Clarified the testing recommendations for anaerobic bacteria (p. 28)

- **Table 2A. Zone Diameter and MIC Breakpoints for *Enterobacteriaceae:***
  - Added references to Tables 4A-2 and 5A-2 for selecting recommended strains for routine QC of β-lactam combination agents (p. 30)

  - Added disk diffusion breakpoints for ceftolozane-tazobactam (p. 31)

  - Added reporting group, disk diffusion and MIC breakpoints, and dosage regimen for ceftazidime-avibactam (p. 31)

  - Clarified the reporting comments for:
    - Cefazolin (when used as a surrogate test for oral cephalosporins and uncomplicated urinary tract infection) (p. 33)
    - Carbapenems (p. 34)
    - Gemifloxacin (p. 35)

  - ***Deleted*** all references to the modified Hodge test

Overview of Changes (Continued)

- **Table 2B-1. Zone Diameter and MIC Breakpoints for *Pseudomonas aeruginosa:***
  - Added:
    - References to Tables 4A-2 and 5A-2 for selecting recommended strains for routine QC of β-lactam combination agents (p. 38)
    - Reporting group, disk diffusion and MIC breakpoints, and dosage regimen for ceftazidime-avibactam (p. 39)
    - Testing method comment for colistin (p. 40)

- **Table 2B-2. Zone Diameter and MIC Breakpoints for *Acinetobacter* spp.:**
  - Added:
    - References to Tables 4A-2 and 5A-2 for selecting recommended strains for routine QC of β-lactam combination agents (p. 42)
    - Testing method comment for colistin (p. 43)

- **Table 2B-3. Zone Diameter and MIC Breakpoints for *Burkholderia cepacia* complex:**
  - Added references to Tables 4A-2 and 5A-2 for selecting recommended strains for routine QC of β-lactam combination agents (p. 46)

- **Table 2B-4. Zone Diameter and MIC Breakpoints for *Stenotrophomonas maltophilia:***
  - Added references to Tables 4A-2 and 5A-2 for selecting recommended strains for routine QC of β-lactam combination agents (p. 48)

- **Table 2B-5. MIC Breakpoints for Other Non-*Enterobacteriaceae:***
  - Added references to Tables 4A-2 and 5A-2 for selecting recommended strains for routine QC of β-lactam combination agents (p. 50)

- **Table 2C. Zone Diameter and MIC Breakpoints for *Staphylococcus* spp.:**
  - Added:
    - *S. schleiferi* to *Staphylococcus pseudintermedius* for oxacillin test interpretation (p. 58)
    - Reporting group and MIC breakpoints for dalbavancin (p. 59)

  - Clarified:
    - Recommendations for detecting oxacillin resistance (p. 55)
    - Oxacillin reporting comments for *S. aureus* and *Staphylococcus lugdunensis* (p. 57)
    - Oxacillin reporting comment for CoNS except *S. lugdunensis, S. pseudintermedius,* and *S. schleiferi* (p. 58)

  - ***Deleted*** all aminoglycoside reporting groups and breakpoints except gentamicin

Overview of Changes (Continued)

- **Table 2D. Zone Diameter and MIC Breakpoints for *Enterococcus* spp.:**
  - Added reporting group, MIC breakpoints, and reporting comment for dalbavancin (p. 66)
  - Clarified reporting comment for *Enterococcus* with low-level penicillin or ampicillin resistance when combination therapy with a β-lactam is being considered (p. 65)
  - ***Deleted*** reporting comment from gatifloxacin to only use for testing and reporting of urinary tract isolates
- **Table 2H-1. Zone Diameter and MIC Breakpoints for *Streptococcus* spp. β-Hemolytic Group:**
  - Added reporting group, MIC breakpoints, and reporting comment for dalbavancin (p. 83)
- **Table 2H-2. Zone Diameter and MIC Breakpoints for *Streptococcus* spp. Viridans Group:**
  - Added reporting group, MIC breakpoints, and reporting comment for dalbavancin (p. 87)
- **Table 2J. MIC Breakpoints for Anaerobes:**
  - Revised susceptible and intermediate breakpoints for piperacillin-tazobactam (p. 95)
- **Introduction to Tables 3B and 3C. Tests for Carbapenemases in *Enterobacteriaceae* and *Pseudomonas aeruginosa:***
  - Added:
    - Text and table information for performing mCIM with eCIM (pp. 102–103)
    - *P. aeruginosa* to test organisms for mCIM (p. 103)
  - ***Deleted:***
    - MHT as a recommended phenotypic method for detecting carbapenemases
    - Modified Hodge test table (for suspected carbapenemase production in *Enterobacteriaceae*) and associated figures
    - Recommendation for testing *Acinetobacter* spp. with the CarbaNP test due to poor sensitivity
- **Table 3B. CarbaNP Test for Suspected Carbapenemase Production in *Enterobacteriaceae* and *Pseudomonas aeruginosa:***
  - ***Deleted*** recommendation for testing *Acinetobacter* spp. and inserted note clarifying this decision
- **Table 3B-1. Modifications of Table 3B When Using MIC Breakpoints for Carbapenems Described in M100-S20 (January 2010):**
  - Reformatted Instructions for Preparation of Test Components into step-action tables (pp. 108–109)

Overview of Changes (Continued)

- **Table 3C. Modified Carbapenem Inactivation Methods for Suspected Carbapenemase Production in *Enterobacteriaceae* and *Pseudomonas aeruginosa:***
  - Added:
    - Recommendation for mCIM testing with *P. aeruginosa* (p. 112)
    - Test procedure, interpretation, reporting recommendations, associated figures, and notes for performing the optional eCIM on mCIM-positive *Enterobacteriaceae* (pp. 113-122)

- **Table 3C-1. Modifications of Table 3C When Using MIC Breakpoints for Carbapenems Described in M100-S20 (January 2010):**
  - ***Deleted*** recommendation for testing *P. aeruginosa*

- **Table 3E. Test for Detection of Methicillin Resistance (Oxacillin Resistance) in *Staphylococcus* species, Except *Staphylococcus pseudintermedius* and *Staphylococcus schleiferi:***
  - Added *S. schleiferi* to the list of organisms to test for oxacillin resistance (p. 130)

- **Table 3I. Test for Detection of High-Level Aminoglycoside Resistance in *Enterococcus* spp. (Includes Disk Diffusion):**
  - Updated susceptible MIC correlates for streptomycin (p. 142)

- **Table 4A-1. Disk Diffusion QC Ranges for Nonfastidious Organisms and Antimicrobial Agents Excluding β-Lactam Combination Agents (p. 144):**
  - Revised title to replace unsupplemented Mueller-Hinton medium with antimicrobial agents excluding β-lactam combination agents

  - Removed all β-lactam combination agents, associated single agents, footnotes, and QC organisms specific for β-lactam combination agents (*E. coli* ATCC® 35218 and *K. pneumoniae* ATCC® 700603) from the table and created a new β-lactam combination agent QC table (see Table 4A-2)

  - Added or changed QC ranges for:
    - *E. coli* ATCC® 25922
      - Cefiderocol
      - Cefixime
      - Ciprofloxacin

    - *P. aeruginosa* ATCC® 27853
      - Cefiderocol

Overview of Changes (Continued)

- **Table 4A-2. Disk Diffusion QC Ranges for Nonfastidious Organisms and β-Lactam Combination Agents (p. 148):**
  - Added new table that includes:
    - All β-lactam combination agents, associated single agents, footnotes, and QC organisms specific for β-lactam combination agents
    - Clarification for when each strain should be tested
    - Specific characteristics for listed QC strains
    - Footnotes specific for β-lactam combination agents

- **Table 4C. Disk Diffusion: Reference Guide to QC Frequency (p. 154):**
  - ***Deleted*** information for converting from daily to weekly QC (refer to M02[1])

- **Table 4D. Disk Diffusion: Troubleshooting Guide (p. 156):**
  - Reorganized antimicrobial agents by drug class
  - Clarified information in the introductory text

- **Table 5A-1. MIC QC Ranges for Nonfastidious Organisms and Antimicrobial Agents Excluding β-Lactam Combination Agents (p. 160):**
  - Revised title to replace unsupplemented Mueller-Hinton medium with antimicrobial agents excluding β-lactam combination agents

  - Removed all β-lactam combination agents, associated single agents, footnotes, and QC organisms specific for β-lactam combination agents (*E. coli* ATCC® 35218 and *K. pneumoniae* ATCC® 700603) from the table and created a new β-lactam combination agent QC table (see Table 5A-2)

  - Added QC ranges for *E. coli* ATCC® 25922 and *P. aeruginosa* ATCC® 27853 for cefiderocol and zidebactam

- **Table 5A-2. MIC QC Ranges for Nonfastidious Organisms and β-Lactam Combination Agents (p. 166):**
  - Added new table that includes:
    - All β-lactam combination agents, associated single agents, footnotes, and QC organisms specific for β-lactam combination agents
    - Clarification for when each strain should be tested
    - Specific characteristics for listed QC strains
    - Footnotes specific for β-lactam combination agents

Overview of Changes (Continued)

- Added QC ranges for:
  - *E. coli* ATCC® 25922:
    - Cefepime-zidebactam
    - Zidebactam
  - *P. aeruginosa* ATCC® 27853:
    - Cefepime-zidebactam
    - Zidebactam
  - *E. coli* ATCC® 35218
    - Meropenem
  - *K. pneumoniae* ATCC® 700603:
    - Cefepime-tazobactam
    - Cefepime-zidebactam
    - Imipenem
    - Imipenem-relebactam
  - *E. coli* NCTC 13353:
    - Cefepime-zidebactam
    - Zidebactam
  - *K. pneumoniae* ATCC® BAA-1705™:
    - Imipenem
  - *K. pneumoniae* ATCC® BAA-2814™:
    - Imipenem
  - *Acinetobacter baumannii* NCTC 13304:
    - Cefepime-zidebactam
    - Zidebactam

Overview of Changes (Continued)

- **Table 5B. MIC QC Ranges for Fastidious Organisms (Broth Dilution Methods) (p. 170):**
  - Added QC ranges for *S. pneumoniae* ATCC® 49619 for imipenem-relebactam
  - ***Deleted*** linopristin-flopristin
- **Table 5D. MIC QC Ranges for Anaerobes (Agar Dilution Method) (p. 176):**
  - Added QC ranges for:
    - *Bacteroides fragilis* ATCC® 25285:
      - Imipenem-relebactam
    - *Bacteroides thetaiotaomicron* ATCC® 29741:
      - Imipenem-relebactam
    - *Clostridioides* (formerly *Clostridium*) *difficile* ATCC® 700057:
      - Ridinilazole
    - *Eggerthella lenta* (formerly *Eubacterium lentum*) ATCC® 43055:
      - Imipenem-relebactam
- **Table 5E. MIC QC Ranges for Anaerobes (Broth Microdilution Method) (p. 178):**
  - Added QC ranges for:
    - *B. fragilis* ATCC® 25285:
      - Imipenem-relebactam
    - *Clostridioides* (formerly *Clostridium*) *difficile* ATCC® 700057:
      - Ridinilazole
- **Table 5F. MIC: Reference Guide to QC Frequency (p. 180):**
  - ***Deleted*** information for converting from daily to weekly QC (see M07[2])
- **Table 5G. MIC: Troubleshooting Guide (p. 182):**
  - Reorganized antimicrobial agents by drug class
  - Added QC strain, *K. pneumoniae* ATCC® 700603, for amoxicillin-clavulanate and ticarcillin-clavulanate

- Clarified comments and suggested action for carbapenems and *P. aeruginosa* when the antimicrobial agent is degrading

- Added QC troubleshooting information for:
  - Chloramphenicol
  - Clindamycin
  - Dalbavancin
  - Erythromycin
  - Linezolid
  - Oritavancin
  - Tedizolid
  - Telavancin
  - Tetracycline
  - Various agents

- **Table 6A. Solvents and Diluents for Preparation of Stock Solutions of Antimicrobial Agents (p. 186):**
  - Added:
    - Cefiderocol
    - Ridinilazole
    - Vaborbactam

  - ***Deleted*** linopristin-flopristin

- **Table 6C. Preparing Solutions and Media Containing Combinations of Antimicrobial Agents (p. 194):**
  - Added:
    - Cefepime-zidebactam
    - Meropenem-vaborbactam

  - ***Deleted*** linopristin-flopristin

Overview of Changes (Continued)

- **Appendix A. Suggestions for Confirming Resistant, Intermediate, or Nonsusceptible Antimicrobial Susceptibility Test Results and Organism Identification:**
  - Changed resistance phenotype detected for colistin/polymyxin from intermediate (I) to non-wild-type (NWT) for any *Enterobacteriaceae* (p. 204)
  - Added "Dalbavancin – NS" for resistance phenotype detected to *Enterococcus* spp. (p. 205); *S. aureus* (p. 206); *Streptococcus,* β-hemolytic group (p. 207); and *Streptococcus,* viridans group (p. 207)
- **Appendix B. Intrinsic Resistance:**
  - Clarified introductory reporting information (p. 210)
  - Added footnote clarifying the species included in the *Enterobacter cloacae* complex (p. 210)
- **Appendix C. QC Strains for Antimicrobial Susceptibility Tests:**
  - Organized organisms alphabetically and by strain number
  - Added:
    - *A. baumannii* NCTC 13304 (p. 216)
    - *K. pneumoniae* ATCC® BAA-2814™ (formerly known as B21(KP1074)), including organism characteristics and testing information (p. 217)
    - Comment for *E. lenta,* noting that testing it is no longer required when establishing new QC ranges (p. 216)
    - Organism characteristics and/or testing information for:
      - *E. coli* ATCC® 35218 (p. 217)
      - *E. coli* NCTC 13353 (p. 217)
      - *K. pneumoniae* ATCC® 700603 (p. 217)
      - *K. pneumoniae* ATCC® BAA-1705™ (p. 217)
      - *K. pneumoniae* ATCC® BAA-1706™ (p. 217)
      - *S. aureus* ATCC® 25923 (p. 218)
      - *S. aureus* ATCC® 29213 (p. 218)
      - *S. aureus* ATCC® BAA-976™ (p. 218)
      - *S. aureus* ATCC® BAA-977™ (p. 218)
  - Clarified language in footnotes (p. 219)
  - ***Deleted*** "Routine" vs "Supplemental" from the column headings (refer to Tables 4A-1, 4A-2, 5A-1 and 5A-2)

Overview of Changes (Continued)

- **Appendix D. Cumulative Antimicrobial Susceptibility Report for Anaerobic Organisms:**
  - Updated antibiogram data for all listed organisms (pp. 222–226)
  - Updated origin of testing data (pp. 223 and 227)
  - Added antibiogram data for penicillin and imipenem for all anaerobic organisms other than *B. fragilis*
  - ***Deleted*** antibiogram data for:
    - *Bacteroides eggerthii*
    - *Veillonella* spp.
    - Cefoxitin and ertapenem for all organisms other than *B. fragilis*

- **Appendix E. Dosing Regimens Used to Establish Susceptible or Susceptible-Dose Dependent Breakpoints:**
  - Added antimicrobial agents and dosage regimens for:
    - Azithromycin (*Salmonella* Typhi) (p. 228)
    - Ceftazidime-avibactam (pp. 228 and 229)
    - Ceftolozane-tazobactam (p. 228)
    - Colistin (p. 229)
    - Dalbavancin (pp. 229 and 230)
    - Meropenem (p. 228)
    - Oritavancin (pp. 229 and 230)
    - Tedizolid (pp. 229 and 230)
    - Telavancin (pp. 229 and 230)

- **Appendix G. Epidemiological Cutoff Values:**
  - Consolidated epidemiological cutoff value (ECV) information originally found in the Instructions for Use of Tables and in the former Tables 2A-2, 2F-2, and 2J-2 (pp. 236–241)
  - Added cautionary text regarding the use and interpretation of ECVs (pp. 236 and 239)
  - Added a testing comment for colistin in Table G1 (p. 239)
  - Revised comment about vancomycin therapy in Table G3 to include both *Cutibacterium* (formerly *Propionibacterium*) *acnes* and *Clostridioides* (formerly *Clostridium*) *difficile* infection (p. 240)

Overview of Changes (Continued)

- **Glossary I (Parts 1 and 2), Glossary II, and Glossary III:**
  - Added explanatory text for the origin of the glossaries

- **Glossary I (Part 1). β-Lactams: Class and Subclass Designations and Generic Name (p. 242):**
  - Added cefepime-zidebactam

- **Glossary I (Part 2). Non–β-Lactams: Class and Subclass Designations and Generic Name (p. 244):**
  - Updated class and subclass designations

  - Added:
    - Nitazoxanide
    - Ramoplanin
    - Retapamulin
    - Rifabutin
    - Rifapentine
    - Thiamphenicol
    - Tizoxanide

  - ***Deleted*** linopristin-flopristin

- **Glossary II. Abbreviations/Routes of Administration/Drug Class for Antimicrobial Agents (p. 248):**
  - Added:
    - Cefepime-zidebactam
    - Cefiderocol

  - ***Deleted*** linopristin-flopristin

NOTE: The content of this document is supported by the CLSI consensus process and does not necessarily reflect the views of any single individual or organization.

Summary of CLSI Processes for Establishing Breakpoints and Quality Control Ranges

The Clinical and Laboratory Standards Institute (CLSI) is an international, voluntary, not-for-profit, interdisciplinary, standards-developing, and educational organization accredited by the American National Standards Institute that develops and promotes the use of consensus-developed standards and guidelines within the health care community. These consensus standards and guidelines are developed in an open and consensus-seeking forum to cover critical areas of diagnostic testing and patient health care. CLSI is open to anyone or any organization that has an interest in diagnostic testing and patient care. Information about CLSI can be found at www.clsi.org.

The CLSI Subcommittee on Antimicrobial Susceptibility Testing reviews data from a variety of sources and studies (eg, *in vitro,* pharmacokinetics-pharmacodynamics, and clinical studies) to establish antimicrobial susceptibility test methods, breakpoints, and QC parameters. The details of the data necessary to establish breakpoints, QC parameters, and how the data are presented for evaluation are described in CLSI document M23.[4]

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

Additional information, updates, and changes in this document are found in the meeting summary minutes of the Subcommittee on Antimicrobial Susceptibility Testing at www.clsi.org.

CLSI Reference Methods vs Commercial Methods and CLSI vs US Food and Drug Administration Breakpoints

It is important for users of M02,[1] M07,[2] and M100 to recognize that the standard methods described in CLSI documents are reference methods. These methods may be used for routine antimicrobial susceptibility testing of patient isolates, for evaluating commercial devices that will be used in medical laboratories, or by drug or device manufacturers for testing new agents or systems. Results generated by reference methods, such as those included in CLSI documents, may be used by regulatory authorities to evaluate the performance of commercial susceptibility testing devices as part of the approval process. Clearance by a regulatory authority indicates the commercial susceptibility testing device provides susceptibility results that are substantially equivalent to results generated using reference methods for the organisms and antimicrobial agents described in the device manufacturer's approved package insert.

CLSI breakpoints may differ from those approved by various regulatory authorities for many reasons, including use of different databases, differences in data interpretation, differences in doses used in different parts of the world, and public health policies. Differences also exist because CLSI proactively evaluates the need for changing breakpoints. The reasons why breakpoints may change and the manner in which CLSI evaluates data and determines breakpoints are outlined in CLSI document M23.[4]

Following a decision by CLSI to change an existing breakpoint, regulatory authorities may also review data to determine how changing breakpoints may affect the safety and effectiveness of the antimicrobial agent for the approved indications. If the regulatory authority changes breakpoints, commercial device manufacturers may have to conduct a clinical trial, submit the data to the regulatory authority, and await review and approval. For these reasons, a delay of one or more years may be needed if a breakpoint and interpretive category change is to be implemented by a device manufacturer. In the United States, it is acceptable for laboratories that use US Food and Drug Administration (FDA)–cleared susceptibility testing devices to use existing FDA breakpoints. Either FDA or CLSI susceptibility breakpoints are acceptable to laboratory accrediting organizations in the United States. Policies in other countries may vary. Each laboratory should check with the manufacturer of its antimicrobial susceptibility test system for additional information on the breakpoints and interpretive categories used in its system's software.

Following discussions with appropriate stakeholders (eg, **infectious diseases and pharmacy practitioners**, the pharmacy and therapeutics and infection control committees of the medical staff, **and the antimicrobial stewardship team)**, newly approved or revised breakpoints may be implemented by laboratories. Following verification, CLSI disk diffusion test breakpoints may be implemented as soon as they are published in M100. If a device includes antimicrobial test concentrations sufficient to allow interpretation of susceptibility and resistance to an agent using the CLSI breakpoints, a laboratory could choose to, after appropriate verification, interpret and report results using CLSI breakpoints.

CLSI Breakpoint Additions/Revisions Since 2010

Antimicrobial Agent	Date of Addition/Revision* (M100 edition)	Comments
Enterobacteriaceae		
Azithromycin – *S.* Typhi only	January 2015 (M100-S25)	
Aztreonam	January 2010 (M100-S20)	
Cefazolin	January 2010 (M100-S20) January 2011 (M100-S21)	Breakpoints were revised twice since 2010.
	January 2014 (M100-S24) January 2016 (M100S, 26th ed.)	Breakpoints were added to predict results for cefazolin when cefazolin is used for therapy of uncomplicated UTIs.
Cefepime	January 2014 (M100-S24)	
Cefotaxime	January 2010 (M100-S20)	
Ceftaroline	January 2013 (M100-S23)	**NPBP**
Ceftazidime	January 2010 (M100-S20)	
Ceftazidime-avibactam	**January 2018 (M100, 28th ed.)**	**NPBP**
Ceftizoxime	January 2010 (M100-S20)	
Ceftolozane-tazobactam	January 2016 (M100S, 26th ed.) **January 2018 (M100, 28th ed.)**	**NPBP** **Disk diffusion breakpoints were added.**
Ceftriaxone	January 2010 (M100-S20)	
Ciprofloxacin – *Salmonella* spp. (including *S.* Typhi)	January 2012 (M100-S22)	Body site–specific breakpoint recommendations were removed in 2013.
Doripenem	June 2010 (M100-S20-U)	**NPBP**
Ertapenem	June 2010 (M100-S20-U) January 2012 (M100-S22)	Breakpoints were revised twice since 2010.
Imipenem	June 2010 (M100-S20-U)	
Levofloxacin – *Salmonella* spp. (including *S.* Typhi)	January 2013 (M100-S23)	
Meropenem	June 2010 (M100-S20-U)	
Ofloxacin – *Salmonella* spp. (including *S.* Typhi)	June 2013 (M100-S23)	
Pefloxacin – *Salmonella* spp. (including *S.* Typhi)	January 2015 (M100-S25)	Surrogate test for ciprofloxacin was added.

CLSI Breakpoint Additions/Revisions Since 2010 (Continued)

Antimicrobial Agent	Date of Addition/Revision* (M100 edition)	Comments
Pseudomonas aeruginosa		
Ceftazidime-avibactam	**January 2018 (M100, 28th ed.)**	**NPBP**
Colistin	January 2017 (M100, 27th ed.)	MIC breakpoints were revised.
Doripenem	January 2012 (M100-S22)	
Imipenem	January 2012 (M100-S22)	
Meropenem	January 2012 (M100-S22)	
Piperacillin	January 2012 (M100-S22)	
Piperacillin-tazobactam	January 2012 (M100-S22)	
Ticarcillin	January 2012 (M100-S22)	
Ticarcillin-clavulanate	January 2012 (M100-S22)	
***Acinetobacter* spp.**		
Doripenem	January 2014 (M100-S24)	
Imipenem	January 2014 (M100-S24)	
Meropenem	January 2014 (M100-S24)	
***Staphylococcus* spp.**		
Ceftaroline	January 2013 (M100-S23)	**NPBP**
Dalbavancin	**January 2018 (M100, 28th ed.)**	**NPBP**
Oritavancin	January 2016 (M100S, 26th ed.)	**NPBP**
Tedizolid	January 2016 (M100S, 26th ed.)	**NPBP**
Telavancin	January 2016 (M100S, 26th ed.)	**NPBP**
***Enterococcus* spp.**		
Dalbavancin	**January 2018 (M100, 28th ed.)**	**NPBP**
Oritavancin	January 2016 (M100S, 26th ed.)	NPBP
Tedizolid	January 2016 (M100S, 26th ed.)	NPBP
Telavancin	January 2016 (M100S, 26th ed.)	NPBP
Haemophilus influenzae* and *Haemophilus parainfluenzae		
Ceftaroline	January 2013 (M100-S23)	**NPBP**
Streptococcus pneumoniae		
Ceftaroline	January 2013 (M100-S23)	**NPBP**
Doxycycline	January 2013 (M100-S23)	**NPBP**
Tetracycline	January 2013 (M100-S23)	

CLSI Breakpoint Additions/Revisions Since 2010 (Continued)

Antimicrobial Agent	Date of Addition/Revision* (M100 edition)	Comments
***Streptococcus* spp. β-Hemolytic Group**		
Ceftaroline	January 2013 (M100-S23)	**NPBP**
Dalbavancin	**January 2018 (M100, 28th ed.)**	**NPBP**
Oritavancin	January 2016 (M100S, 26th ed.)	**NPBP**
Telavancin	January 2016 (M100S, 26th ed.)	**NPBP**
***Streptococcus* spp. Viridans Group**		
Ceftolozane-tazobactam	January 2016 (M100S, 26th ed.)	**NPBP**
Dalbavancin	**January 2018 (M100, 28th ed.)**	**NPBP**
Oritavancin	January 2016 (M100S, 26th ed.)	**NPBP**
Tedizolid	January 2016 (M100S, 26th ed.)	**NPBP**
Telavancin	January 2016 (M100S, 26th ed.)	**NPBP**

* Previous breakpoints can be found in the edition of M100 that precedes the document listed here, eg, previous breakpoints for aztreonam are listed in M100-S19 (January 2009).
Abbreviations: MIC, minimal inhibitory concentration; **NPBP, no previous breakpoint existed;** UTI, urinary tract infection.

CLSI Epidemiological Cutoff Value Additions/Revisions Since 2015

Antimicrobial Agent	Date of Addition/Revision (M100 edition)	Comments
Enterobacteriaceae		
Azithromycin	January 2016 (M100S, 26th ed.)	For use with *Shigella flexneri* and *Shigella sonnei*.
Colistin	January 2017 (M100, 27th ed.)	For use with ***Klebsiella*** **(formerly** *Enterobacter***)** *aerogenes, E. cloacae, E. coli, K. pneumoniae,* and *Raoultella ornithinolytica.*
Neisseria gonorrhoeae		
Azithromycin	January 2017 (M100, 27th ed.)	
Anaerobes		
Piperacillin-tazobactam	**January 2018 (M100, 28th ed.)**	**MIC breakpoints were revised.**
Vancomycin	January 2015 (M100-S25)	For use with ***Cutibacterium*** **(formerly** *Propionibacterium***)** *acnes*.

Abbreviation: MIC, minimal inhibitory concentration.

CLSI Archived Resources

Resource	Web Address for Archived Table
Breakpoints that have been eliminated from M100 since 2010 have been relocated to the CLSI website.	https://clsi.org/media/1828/_m100_archived_drugs_table.pdf
Methods that have been eliminated from M100 have been relocated to the CLSI website.	https://clsi.org/media/1899/_m100_archived_methods_table.pdf

Subcommittee on Antimicrobial Susceptibility Testing Mission Statement

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

- Develop standard reference methods for antimicrobial susceptibility tests.
- Provide quality control parameters for standard test methods.
- Establish breakpoints for the results of standard antimicrobial susceptibility tests and provide epidemiological cutoff values when breakpoints are not available.
- Provide suggestions for testing and reporting strategies that are clinically relevant and cost-effective.
- Continually refine standards and optimize detection of emerging resistance mechanisms through development of new or revised methods, breakpoints, and quality control parameters.
- Educate users through multimedia communication of standards and guidelines.
- Foster a dialogue with users of these methods and those who apply them.

The ultimate purpose of the subcommittee's mission is to provide useful information to enable laboratories to assist the clinician in the selection of appropriate antimicrobial therapy for patient care. The standards and guidelines are meant to be comprehensive and to include all antimicrobial agents for which the data meet established CLSI guidelines. The values that guide this mission are quality, accuracy, fairness, timeliness, teamwork, consensus, and trust.

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Instructions for Use of Tables

These instructions apply to:

- **Tables 1A and 1B:** suggested groupings of antimicrobial agents that should be considered for testing and reporting by microbiology laboratories. These guidelines are based on antimicrobial agents approved by the US Food and Drug Administration (FDA) for clinical use in the United States. In other countries, placement of antimicrobial agents in Tables 1A and 1B should be based on available drugs approved for clinical use by relevant regulatory organizations.

- **Tables 2A through 2I:** tables for each organism group that contain:
  - Recommended testing conditions
  - Routine QC recommendations (also see Chapter 4 in M02[1] and M07[2])
  - General comments for testing the organism group and specific comments for testing particular agent/organism combinations
  - Suggested agents that should be considered for routine testing and reporting by medical microbiology laboratories, as specified in Tables 1A and 1B (test/report groups A, B, C, U)
  - Additional drugs that have an approved indication for the respective organism group but would generally not warrant routine testing by a medical microbiology laboratory in the United States (test/report group O for "other"; test/report group Inv. for "investigational" [not yet FDA approved])
  - Zone diameter and minimal inhibitory concentration (MIC) breakpoints

- **Tables 1C and 2J:** tables containing specific recommendations for testing and reporting results on anaerobes and some of the information listed in the bullets above

- **Tables 3A to 3I:** tables describing tests to detect particular resistance types in specific organisms or organism groups

I. Selecting Antimicrobial Agents for Testing and Reporting

A. Selecting the most appropriate antimicrobial agents to test and report is a decision best made by each laboratory in consultation with the **infectious diseases and pharmacy practitioners**, the pharmacy and therapeutics and infection control committees of the medical staff, **and the antimicrobial stewardship team**. The recommendations for each organism group include 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 use, and current consensus recommendations for first-choice and alternative drugs. Tests of selected agents may be useful for infection control purposes.

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B. Drugs listed together in a single box are agents for which interpretive categories (susceptible, intermediate, or resistant) and clinical efficacy are similar. Within each box, an "or" between agents indicates agents for which cross-resistance and cross-susceptibility are nearly complete. Results from one agent connected by an "or" can be used to predict results for the other agent. For example, *Enterobacteriaceae* susceptible to cefotaxime can be considered susceptible to ceftriaxone. The results obtained from testing cefotaxime could be reported along with a comment that the isolate is also susceptible to ceftriaxone. For drugs connected with an "or," combined major and very major errors are fewer than 3%, and minor errors are fewer than 10%, based on a large population of bacteria tested (see CLSI document M23[4] for description of error types). In addition, to qualify for an "or," at least 100 strains with resistance to the agents in question must be tested, and a result of "resistant" must be obtained with all agents for at least 95% of the strains. "Or" is also used for comparable agents when tested against organisms for which "susceptible-only" breakpoints are provided (eg, cefotaxime or ceftriaxone with *H. influenzae*). When no "or" connects agents within a box, testing of one agent cannot be used to predict results for another, owing either to discrepancies or insufficient data.

C. Test/Report Groups

1. As listed in Tables 1A, 1B, and 1C, agents in **group A** are considered appropriate for inclusion in a routine, primary testing panel, as well as for routine reporting of results for the specific organism groups.

2. **Group B** includes antimicrobial agents that may warrant primary testing, but they may be reported only selectively, such as when the organism is resistant to agents of the same antimicrobial class, as in group A. Other indications for reporting the result might include a selected specimen source (eg, a third-generation cephalosporin for enteric bacilli from 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 antimicrobial agent in group A; or for infection control purposes.

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

4. **Group U ("urine")** includes certain antimicrobial agents (eg, nitrofurantoin and certain quinolones) that are used only or primarily for treating UTIs. These agents should not be routinely reported against pathogens recovered from other infection sites. An exception to this rule is for *Enterobacteriaceae* in Table 1A, in which cefazolin is listed as a surrogate agent for oral cephalosporins. Other antimicrobial agents with broader indications may be included in group U for specific urinary pathogens (eg, *Enterococcus* and ciprofloxacin).

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

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6. **Group Inv. ("investigational")** includes antimicrobial agents that are investigational for the organism group and have not yet been approved by the FDA for use in the United States.

D. Selective Reporting

Each laboratory should decide which agents in the tables to report routinely (group A) and which might be reported only selectively (from group B), in consultation with the infectious diseases **and** pharmacy **practitioners**, the pharmacy and therapeutics and infection control committees of the health care institution, **and the antimicrobial stewardship team**. Selective reporting should improve the clinical relevance of test reports and help minimize the selection of multiresistant, health care–associated strains by overusing broad-spectrum antimicrobial agents. Results for group B antimicrobial agents tested, but not reported routinely, should be available on request, or they may be reported for selected specimen types. Unexpected resistance, when confirmed, should be reported (eg, resistance to a secondary agent but susceptibility to a primary agent, such as a *P. aeruginosa* isolate resistant to amikacin but susceptible to tobramycin; as such, both drugs should be reported). In addition, each laboratory should develop a protocol to cover isolates that are confirmed as resistant to all agents on its routine test panels. This protocol should include options for testing additional agents in-house or sending the isolate to a referral laboratory.

II. Breakpoint and Interpretive Category Definitions

A. **Breakpoint** – minimal inhibitory concentration (MIC) or zone diameter value used to categorize an organism as susceptible, susceptible-dose dependent, intermediate, resistant, or nonsusceptible; **NOTE 1:** MIC or zone diameter values generated by a susceptibility test can be interpreted based upon established breakpoints; **NOTE 2:** Because breakpoints are based on pharmacologically and clinically rich datasets using *in vitro* and *in vivo* data, they are considered robust predictors of likely clinical outcome; **NOTE 3:** Also known as "clinical breakpoint"; **NOTE 4:** See **interpretive category.**

B. **Interpretive category** – category derived from microbiology characteristics, pharmacokinetic-pharmacodynamic parameters, and clinical outcome data, when available; **NOTE 1:** MIC or zone diameter values generated by a susceptibility test can be interpreted based upon established breakpoints; **NOTE 2:** See **breakpoint.**

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EXAMPLE:

Interpretive Category	Breakpoints*	
	MIC, µg/mL	Zone Diameter, mm
Susceptible	≤4	≥20
Susceptible-dose dependent	8–16	15–19
Intermediate	8–16	15–19
Resistant	≥32	≤14
Nonsusceptible	>4	<20

* Formerly "interpretive criteria."

MIC or zone diameter value breakpoints and interpretive categories are established per CLSI document M23[4] for categories of susceptible, intermediate, and resistant (and susceptible-dose dependent and nonsusceptible, when appropriate).

- **Susceptible (S)** – a category defined by a breakpoint that implies that isolates with an MIC at or below or zone diameters at or above the susceptible breakpoint are inhibited by the usually achievable concentrations of antimicrobial agent when the dosage recommended to treat the site of infection is used, resulting in likely clinical efficacy.

- **Susceptible-dose dependent (SDD)** – a category defined by a breakpoint that implies that susceptibility of an isolate is dependent on the dosing regimen that is used in the patient. In order to achieve levels that are likely to be clinically effective against isolates for which the susceptibility testing results (either minimal inhibitory concentrations [MICs] or zone diameters) are in the SDD category, it is necessary to use a dosing regimen (ie, higher doses, more frequent doses, or both) that results in higher drug exposure than the dose that was used to establish the susceptible breakpoint. Consideration should be given to the maximum approved dosage regimen, because higher exposure gives the highest probability of adequate coverage of an SDD isolate. The drug label should be consulted for recommended doses and adjustment for organ function; **NOTE:** The concept of SDD has been included within the intermediate category definition for antimicrobial agents. However, this is often overlooked or not understood by clinicians and microbiologists when an intermediate result is reported. The SDD category may be assigned when doses well above those used to calculate the susceptible breakpoint are approved and used clinically and for which sufficient data to justify the designation exist and have been reviewed. When the intermediate category is used, its definition remains unchanged. See Appendix F for additional information.

- **Intermediate (I)** – a category defined by a breakpoint that includes isolates with MICs or zone diameters within the intermediate range that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates; **NOTE:** The intermediate category implies clinical efficacy in body sites where the drugs are physiologically concentrated or when a higher than normal dosage of a drug can be used. 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.

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- **Resistant (R)** – a category defined by a breakpoint that implies that isolates with an MIC at or above or zone diameters at or below the resistant breakpoint are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules and/or that demonstrate MICs or zone diameters that fall in the range in which specific microbial resistance mechanisms are likely, and clinical efficacy of the agent against the isolate has not been reliably shown in treatment studies.

- **Nonsusceptible (NS)** – a category used for isolates for which only a susceptible breakpoint is designated because of the absence or rare occurrence of resistant strains. Isolates for which the antimicrobial agent MICs are above or zone diameters below the value indicated for the susceptible breakpoint should be reported as nonsusceptible; **NOTE 1:** An isolate that is interpreted as nonsusceptible does not necessarily mean that the isolate has a resistance mechanism. It is possible that isolates with MICs above the susceptible breakpoint that lack resistance mechanisms may be encountered within the wild-type distribution subsequent to the time the susceptible-only breakpoint was set; **NOTE 2:** The term "nonsusceptible" should not be used when describing an organism/drug category with intermediate and resistant interpretive categories. Isolates that are in the categories of "intermediate" or "resistant" could be called "not susceptible" rather than "nonsusceptible."

C. Example of Breakpoints and Interpretive Categories as Used in Table 2

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL		
		S	I*	R	S	I*	R
X	30 µg	≥20	15–19	≤14	≤4	8–16	≥32
Y	–	–	–	–	≤1	2	≥4
Z	10 µg	≥16	–	–	≤1	–	–

* Or SDD, if appropriate.

For antimicrobial agent X with breakpoints in the table above, the susceptible breakpoint is ≤ 4 µg/mL or ≥ 20 mm and the resistant breakpoint is ≥32 µg/mL or ≤14 mm. For some antimicrobial agents (eg, antimicrobial agent Y), only MIC breakpoints may be available. For these agents, the disk diffusion zone diameters do not correlate with MIC values. Technical issues may also preclude the use of the disk diffusion method for some agents. For some antimicrobial agents (eg, antimicrobial agent Z) only a "susceptible" category exists. For these agents, the absence or rare occurrence of resistant strains precludes defining any results categories other than "susceptible." For

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strains yielding results suggestive of a "nonsusceptible" category, organism identification and antimicrobial susceptibility test results should be confirmed (see Appendix A).

In examples Y and Z, a dash mark (–) indicates a disk is not available or that breakpoints are not applicable.

III. Reporting Results

A. Organisms Included in Table 2

The MIC values determined as described in M07[2] may be reported directly to clinicians for patient care purposes. However, it is essential that an interpretive category result (S, I, or R) also be provided routinely to facilitate understanding of the MIC report by clinicians. Zone diameter measurements without an interpretive category should not be reported. Recommended interpretive categories for various MIC and zone diameter values are included in tables for each organism group and are based on the evaluation of data as described in CLSI document M23.[4]

Laboratories should only report results for agents listed in Table 2 specific to the organism being tested. It is not appropriate to apply disk diffusion or MIC breakpoints borrowed from a table in which the organism is not listed. There may be rare cases for which an agent may be appropriate for an isolate but for which there are no CLSI breakpoints (eg, tigecycline). In these cases, the FDA prescribing information document for the agent should be consulted.

For more information on reporting epidemiological cutoff values in the medical laboratory, see Appendix G.

B. Organisms Excluded From Table 2

For some organism groups excluded from Tables 2A through 2J, CLSI document M45[5] provides suggestions for standardized methods for antimicrobial susceptibility testing (AST), including information about drug selection, interpretation, and QC. The organism groups covered in that guideline are *Abiotrophia* and *Granulicatella* spp. (formerly known as nutritionally deficient or nutritionally variant streptococci); *Aerococcus* spp.; *Aeromonas* spp.; *Bacillus* spp. (not *Bacillus anthracis*); *Campylobacter jejuni/coli; Corynebacterium* spp. (including *Corynebacterium diphtheriae*); *Erysipelothrix rhusiopathiae; Gemella* spp.; the HACEK group: *Aggregatibacter* spp. (formerly *Haemophilus aphrophilus, Haemophilus paraphrophilus, Haemophilus segnis,* and *Actinobacillus actinomycetemcomitans*), *Cardiobacterium* spp., *Eikenella corrodens,* and *Kingella* spp.; *Helicobacter pylori; Lactobacillus* spp.; *Lactococcus* spp.; *Leuconostoc* spp.; *Listeria monocytogenes; Micrococcus* spp.; *Moraxella catarrhalis; Pasteurella* spp.; *Pediococcus* spp.; *Rothia mucilaginosa;* potential agents of bioterrorism; and *Vibrio* spp., including *Vibrio cholerae.*

For organisms other than those in the groups mentioned above, studies are not yet adequate to develop reproducible, definitive standards to interpret results. These organisms may need different media or different incubation atmospheres, or they may show marked strain-to-strain variation in growth rate. For these microorganisms, consultation with an infectious diseases specialist is recommended for guidance

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in determining the need for susceptibility testing and in results interpretation. Published reports in the medical literature and current consensus recommendations for therapy of uncommon microorganisms may preclude the need for testing. If necessary, a dilution method usually is the most appropriate testing method, and this may necessitate submitting the organism to a referral laboratory. Physicians should be informed of the limitations of results and advised to interpret results with caution.

C. Cumulative Antibiograms

Policies regarding the generation of cumulative antibiograms should be developed together with the infectious diseases service, infection control personnel, the pharmacy and therapeutics committee, **and the antimicrobial stewardship team**. In most circumstances, the percentage of susceptible and intermediate results should not be combined into the same statistics. See CLSI document M39[6] **for detailed instructions on generating cumulative antibiograms**.

IV. Therapy-Related Comments

Some of the comments in the tables relate to therapy concerns. These are denoted with an ***Rx*** symbol. It may be appropriate to include some of these comments (or modifications thereof) on the patient report. An example would be inclusion of a comment **when rifampin is being reported stating that "Rifampin should not be used alone for antimicrobial therapy."**

Antimicrobial dosage regimens often vary widely among practitioners and institutions. In some cases, the MIC breakpoints rely on pharmacokinetic-pharmacodynamic data, using specific human dosage regimens. In cases in which specific dosage regimens are important for properly applying breakpoints, the dosage regimen is listed. These dosage regimen comments are not generally intended for use on individual patient reports.

V. Confirmation of Patient Results

Multiple test parameters are monitored by following the QC recommendations described in M100. However, acceptable results derived from testing QC strains do not guarantee accurate results when testing patient isolates. It is important to review all of the results obtained from all drugs tested on a patient's isolate before reporting the results. This review should include but not be limited to ensuring that 1) the antimicrobial susceptibility test results are consistent with the identification of the isolate; 2) the results from individual agents within a specific drug class follow the established hierarchy of activity rules (eg, in general, third-generation cephems are more active than first- or second-generation cephems against *Enterobacteriaceae*); and 3) the isolate is susceptible to those agents for which resistance has not been documented (eg, vancomycin and *Streptococcus* spp.) and for which only "susceptible" breakpoints exist in M100.

Unusual or inconsistent results should be confirmed by rechecking various testing parameters detailed in Appendix A. Each laboratory must develop its own policies for confirming unusual or inconsistent antimicrobial susceptibility test results. The list provided in Appendix A emphasizes results that are most likely to affect patient care.

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VI. Development of Resistance and Testing of Repeat Isolates

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

In certain circumstances, the decision to perform susceptibility tests on subsequent isolates necessitates knowledge of the specific situation and the severity of the patient's condition (eg, an isolate of *E. cloacae* from a blood culture on a premature infant or methicillin-resistant *S. aureus* [MRSA] from a patient with prolonged bacteremia). Laboratory guidelines on when to perform susceptibility testing on repeat isolates should be determined after consultation with the medical staff.

VII. Warning

Some of the comments in the tables relate to dangerously misleading results that can occur when certain antimicrobial agents are tested and reported as susceptible against specific organisms. These are denoted with the word **"Warning."**

Location	Organism	Antimicrobial Agents
"Warning": The following antimicrobial agent/organism combinations may appear active *in vitro,* but are not effective clinically and must not be reported as susceptible.		
Table 2A	*Salmonella* spp., *Shigella* spp.	1st- and 2nd-generation cephalosporins, cephamycins, and aminoglycosides
Table 2D	*Enterococcus* spp.	Aminoglycosides (except for high-level resistance testing), cephalosporins, clindamycin, and trimethoprim-sulfamethoxazole
"Warning": The following antimicrobial agents that are included in this document should not be routinely reported for bacteria isolated from CSF. These antimicrobial agents are not the drugs of choice and may not be effective for treating CSF infections caused by these organisms (ie, the bacteria included in Tables 2A through 2J):		
Tables 2A through 2J	**Bacteria isolated from CSF**	**Agents administered by oral route only, 1st- and 2nd-generation cephalosporins and cephamycins, clindamycin, macrolides, tetracyclines, and fluoroquinolones**

Abbreviation: CSF, cerebrospinal fluid.

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VIII. Routine, Supplemental, Screening, Surrogate Agent, and Equivalent Agent Testing to Determine Susceptibility and Resistance to Antimicrobial Agents

The testing categories are defined as follows:

- **Routine test:** disk diffusion or broth or agar dilution MIC tests for routine clinical testing

- **Supplemental (not routine) test:** test that detects susceptibility or resistance to a drug or drug class by method other than routine disk diffusion or broth or agar dilution MIC and does not need additional tests to confirm susceptibility or resistance
  - Some supplemental tests identify a specific resistance mechanism **and** may be required or optional for reporting **specific** clinical results.

- **Screening test:** test that provides presumptive results; additional testing typically only needed for a specific result (eg, only if screen is positive)

- **Surrogate agent test:** test performed with an agent that replaces a test performed with the antimicrobial agent of interest and is used when the agent of interest cannot be tested due to availability or performance issues (eg, surrogate agent performs better than the agent of interest)

- **Equivalent agent test:** test performed with an agent that predicts results of closely related agents of the same class and increases efficiency by limiting testing of multiple closely related agents. Equivalent agents are identified by:
  - Listing equivalent agents with an "or" in Tables 1 and 2. "Or" indicates cross-susceptibility and cross-resistance is nearly complete (very major error + major error < 3%; minor error < 10%) and only one agent needs to be tested.

  - Listing agents that are equivalent and results that can be deduced by testing the equivalent agent in a comment (see Tables 1 and 2).

The following tables include tests that fall into the supplemental, screening, surrogate agent, and equivalent agent test categories. The tables for supplemental, screening, and surrogate agent tests are comprehensive. The table for equivalent agent tests includes several examples, and many other equivalent agent tests are described throughout Tables 1 and 2.

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Supplemental Tests – Required

Supplemental Test	Organisms	Test Description	Required for:	Table Location
Inducible clindamycin resistance	• *S. aureus* • CoNS • *S. pneumoniae* • *Streptococcus* spp. β-hemolytic Group	Broth microdilution or disk diffusion with clindamycin and erythromycin tested together	Isolates that test erythromycin resistant and clindamycin susceptible or intermediate before reporting the isolate as clindamycin susceptible	**3G**
β-lactamase	• CoNS	Chromogenic cephalosporin	Isolates that test penicillin susceptible before reporting the isolate as penicillin susceptible	**3D**
β-lactamase	• *S. aureus*	Chromogenic cephalosporin; penicillin disk diffusion zone-edge test	Isolates that test penicillin susceptible before reporting the isolate as penicillin susceptible	**3D**

Abbreviation: CoNS, coagulase-negative staphylococci.

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Supplemental Tests – Optional

Supplemental Test	Organisms	Test Description	Optional for:	Table Location
ESBL	• *E. coli* • *K. pneumoniae* • *Klebsiella oxytoca* • *Proteus mirabilis*	Broth microdilution or disk diffusion clavulanate inhibition test for ESBLs	Isolates demonstrating reduced susceptibility to cephalosporins Results that indicate presence or absence of ESBLs	3A
CarbaNP	• *Enterobacteriaceae* • *P. aeruginosa*	Colorimetric assay for detecting carbapenem hydrolysis	Isolates demonstrating reduced susceptibility to carbapenems Results that indicate presence or absence of certain carbapenemases	**3B**, **3B**-1
mCIM **with or without eCIM**	• **mCIM only:** *Enterobacteriaceae* **and** ***P. aeruginosa*** • **mCIM with eCIM:** ***Enterobacteriaceae*** **only**	Disk diffusion for detecting carbapenem hydrolysis (inactivation) **eCIM add-on enables differentiation of metallo-β-lactamases from serine carbapenemases in *Enterobacteriaceae* isolates that are positive for mCIM**	Isolates demonstrating reduced susceptibility to carbapenems Results that indicate presence or absence of certain carbapenemases	**3C**
Oxacillin salt agar	• *S. aureus*	Agar dilution; MHA with 4% NaCl and 6 µg/mL oxacillin	Detecting MRSA; see cefoxitin surrogate agent tests, which are preferred	**3E**

Abbreviations: **eCIM, EDTA-modified carbapenem inactivation method;** ESBL, extended-spectrum β-lactamase; mCIM, modified carbapenem inactivation method; MHA, Mueller-Hinton agar; MRSA, methicillin-resistant *Staphylococcus aureus*.

Screening Tests

Screening Test	Organisms	Test Description	When to Perform Confirmatory Test	Confirmatory Test	Table Location
Vancomycin agar screen	• *S. aureus* • *Enterococcus* spp.	Agar dilution; BHI with 6 µg/mL vancomycin	If screen positive	Vancomycin MIC	**3F**
HLAR by disk diffusion	• *Enterococcus* spp.	Disk diffusion with gentamicin and streptomycin	If screen inconclusive	Broth microdilution, agar dilution MIC	**3I**

Abbreviations: BHI, brain heart infusion; HLAR, high-level aminoglycoside resistance; MIC, minimal inhibitory concentration.

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Surrogate Agent Tests

Surrogate Agent	Organisms	Test Description	Results	Table Location
Cefoxitin	• *S. aureus* • *S. lugdunensis* • CoNS • Not for *S. pseudintermedius* **or *S. schleiferi***	Broth microdilution (*S. aureus* and *S. lugdunensis* only) or disk diffusion	Predicts results for *mecA*-mediated oxacillin resistance **NOTE: Some non-*S. epidermidis* strains for which the oxacillin MICs are 0.5–2 µg/mL lack *mecA*. Non-*S. epidermidis* isolates from serious infections with MICs in this range may be tested for *mecA* or for PBP2a. Isolates that test *mecA* or PBP2a negative should be reported as oxacillin susceptible.**	1A, 2C
Cefazolin	• *E. coli* • *Klebsiella* spp. • *P. mirabilis*	Broth microdilution or disk diffusion	When used for therapy of uncomplicated UTIs, predicts results for the following oral antimicrobial agents: cefaclor, cefdinir, cefpodoxime, cefprozil, cefuroxime, cephalexin, and loracarbef **Cefazolin as a surrogate may overcall resistance to cefdinir, cefpodoxime, and cefuroxime. If cefazolin tests resistant, test these drugs individually if needed for therapy**.	1A, 2A
Pefloxacin	• *Salmonella* spp.	Disk diffusion	Predicts reduced susceptibility to ciprofloxacin	2A
Oxacillin	• *S. pneumoniae*	Disk diffusion	Predicts penicillin susceptibility if oxacillin zone is ≥20 mm. If oxacillin zone is ≤19 mm, penicillin MIC must be done.	1B, 2G

Abbreviations: CoNS, coagulase-negative staphylococci; MIC, minimal inhibitory concentration; PBP2a, penicillin-binding protein 2a; UTI, urinary tract infection.

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Examples of Equivalent Agent Tests

Agents	Organisms	Identified by	Table Location
Cefotaxime or ceftriaxone	*Enterobacteriaceae*	"Or"	1A and 2A
Azithromycin or clarithromycin or erythromycin	*Staphylococcus* spp.	"Or"	1A and 2C
Penicillin-susceptible staphylococci are susceptible to other β-lactam agents with established clinical efficacy for staphylococcal infections (including both penicillinase-labile and penicillinase-stable agents; see Glossary I). Penicillin-resistant staphylococci are resistant to penicillinase-labile penicillins.	*Staphylococcus* spp.	Note listed	1A and 2C
The results of ampicillin susceptibility tests should be used to predict the activity of amoxicillin.	*Haemophilus* spp.	Note listed	1B and 2E
The results of ampicillin susceptibility tests should be used to predict the activity of amoxicillin.	**Anaerobes**	**Note listed**	**2J**

IX. Quality Control and Verification

Recommendations for QC are included in various tables and appendixes. Acceptable ranges for QC strains are provided in Tables 4A**-1 through** 4B for disk diffusion and Tables 5A**-1** through 5E for MIC testing. Guidance for QC frequency and modifications of AST systems is found in Table 4C for disk diffusion and Table 5F for MIC testing. Guidance for troubleshooting out-of-range results is included in Table 4D for disk diffusion and Table 5G for MIC testing. Additional information is available in Appendix C (eg, QC organism characteristics, QC testing recommendations).

Implementing any new diagnostic test requires verification.[7] Each laboratory that introduces a new AST system or adds a new antimicrobial agent to an existing AST system must verify or establish that, before reporting patient test results, the system meets performance specifications for that system. Verification generally involves testing patient isolates with the new AST system and comparing results to those obtained with an established reference method or a system that has been previously verified. Testing patient isolates may be done concurrently with the two systems. Alternatively, organisms with known MICs or zone sizes may be used for the verification. Guidance on verification studies is not included in this document. Other publications describe AST system verification (eg, CLSI document M52[8] and Patel J, et al.[9]).

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X. Abbreviations and Acronyms

AST	antimicrobial susceptibility testing
ATCC®[a]	American Type Culture Collection
BHI	brain heart infusion
BLNAR	β-lactamase negative, ampicillin-resistant
BSC	biological safety cabinet
BSL-2	biosafety level 2
BSL-3	biosafety level 3
CAMHB	cation-adjusted Mueller-Hinton broth
CBA	**colistin base activity**
CFU	colony-forming unit(s)
CMRNG	chromosomally mediated penicillin-resistant *Neisseria gonorrhoeae*
CoNS	coagulase-negative staphylococci
CSF	cerebrospinal fluid
DMSO	dimethyl sulfoxide
ECV	epidemiological cutoff value
eCIM	**EDTA-modified carbapenem inactivation method**
EDTA	**ethylenediaminetetraacetic acid**
ESBL	extended-spectrum β-lactamase
FDA	US Food and Drug Administration
HLAR	high-level aminoglycoside resistance
HTM	*Haemophilus* test medium
I	intermediate
ID	identification
KPC	*Klebsiella pneumoniae* carbapenemase
LHB	lysed horse blood
mCIM	modified carbapenem inactivation method
MHA	Mueller-Hinton agar
MHB	Mueller-Hinton broth
MIC	minimal inhibitory concentration
MRS	methicillin-resistant staphylococci

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

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MRSA	methicillin-resistant *Staphylococcus aureus*
NAD	nicotinamide adenine dinucleotide
NCTC	National Collection of Type Cultures
NDM	New Delhi metallo-β-lactamase
NPBP	**no previous breakpoint existed**
NS	nonsusceptible
NWT	non-wild-type
PBP2a	penicillin-binding protein 2a
PCR	polymerase chain reaction
PK-PD	pharmacokinetic-pharmacodynamic
QC	quality control
R	resistant
S	susceptible
SDD	susceptible-dose dependent
TSA	tryptic soy agar
TSB	trypticase soy broth
UTI	urinary tract infection
WT	wild-type

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Table 1A. Suggested Groupings of Antimicrobial Agents Approved by the US Food and Drug Administration for Clinical Use That Should Be Considered for Testing and Reporting on Nonfastidious Organisms by Microbiology Laboratories in the United States

	Enterobacteriaceae	*Pseudomonas aeruginosa*	*Staphylococcus* spp.	*Enterococcus* spp.[m]
GROUP A PRIMARY TEST AND REPORT	Ampicillin[c]	Ceftazidime	Azithromycin[b] or clarithromycin[b] or erythromycin[b]	Ampicillin[n] Penicillin[o]
	Cefazolin[d]	Gentamicin Tobramycin		
	Gentamicin[c] Tobramycin[c]	Piperacillin-tazobactam	Clindamycin[b]	
			Oxacillin[i,k,*,†,§] Cefoxitin[i,k,†] (surrogate test for oxacillin)	
			Penicillin[i]	
			Trimethoprim-sulfamethoxazole	
GROUP B OPTIONAL PRIMARY TEST REPORT SELECTIVELY	Amikacin[c]	Amikacin	Ceftaroline[h]	Daptomycin[j,*]
	Amoxicillin-clavulanate Ampicillin-sulbactam **Ceftazidime-avibactam** Ceftolozane-tazobactam Piperacillin-tazobactam	Aztreonam	Daptomycin[j,*]	Linezolid Tedizolid[p]
		Cefepime	Linezolid Tedizolid[h]	
		Ceftazidime-avibactam Ceftolozane-tazobactam		Vancomycin
	Cefuroxime	Ciprofloxacin Levofloxacin	Doxycycline Minocycline[b] Tetracycline[a]	
	Cefepime	Doripenem Imipenem Meropenem		
	Cefotetan Cefoxitin		Vancomycin[*]	
	Cefotaxime[c,d] or ceftriaxone[c,d]			
	Ciprofloxacin[c] Levofloxacin[c]		Rifampin[g]	
	Doripenem Ertapenem Imipenem Meropenem			
	Trimethoprim-sulfamethoxazole[c]			

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Table 1A. (Continued)

	Enterobacteriaceae	*Pseudomonas aeruginosa*	*Staphylococcus* spp.	*Enterococcus* spp.[m]
GROUP C SUPPLEMENTAL REPORT SELECTIVELY	Aztreonam Ceftazidime		Chloramphenicol[b]	Gentamicin (high-level resistance testing only)
	Ceftaroline		Ciprofloxacin or levofloxacin	Streptomycin (high-level resistance testing only)
	Chloramphenicol[b,c]		Moxifloxacin	**Dalbavancin[r,*]**
	Tetracycline[a]		Gentamicin[l]	Oritavancin[r,*]
			Dalbavancin[h,*]	Telavancin[r,*]
			Oritavancin[h,*]	
			Telavancin[h,*]	
GROUP U SUPPLEMENTAL FOR URINE ONLY	Cefazolin (surrogate test for uncomplicated UTI)‡		Nitrofurantoin	Ciprofloxacin Levofloxacin
	Fosfomycin[e]		Sulfisoxazole	Fosfomycin[q]
	Nitrofurantoin		Trimethoprim	Nitrofurantoin
	Sulfisoxazole			Tetracycline[a]
	Trimethoprim			

* MIC testing only; disk diffusion test unreliable.

† See oxacillin and cefoxitin comments in Table 2C for using cefoxitin as a surrogate for oxacillin.

‡ See cefazolin comments in Table 2A for using cefazolin as a surrogate for oral cephalosporins and for reporting cefazolin when used for therapy in uncomplicated UTIs.

§ **MIC testing only for *S. aureus* and most CoNS; see exceptions in Table 2C.**

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Table 1A. (Continued)

	Acinetobacter spp.	*Burkholderia cepacia* complex	*Stenotrophomonas maltophilia*	Other Non-*Enterobacteriaceae*[f,*]
GROUP A PRIMARY TEST AND REPORT	Ampicillin-sulbactam	Levofloxacin[*]	Trimethoprim-sulfamethoxazole	Ceftazidime
	Ceftazidime	Meropenem		Gentamicin Tobramycin
	Ciprofloxacin Levofloxacin	Trimethoprim-sulfamethoxazole		
	Doripenem Imipenem Meropenem			
	Gentamicin Tobramycin			
GROUP B OPTIONAL PRIMARY TEST REPORT SELECTIVELY	Amikacin	Ceftazidime	Ceftazidime[*]	Amikacin
	Piperacillin-tazobactam	Minocycline	Levofloxacin	Aztreonam
	Cefepime		Minocycline	Cefepime
	Cefotaxime Ceftriaxone			Ciprofloxacin Levofloxacin
	Doxycycline			Imipenem Meropenem
	Minocycline			Piperacillin-tazobactam
	Trimethoprim-sulfamethoxazole			Trimethoprim-sulfamethoxazole
GROUP C SUPPLEMENTAL REPORT SELECTIVELY		Chloramphenicol[b,*]	Chloramphenicol[b,*]	Cefotaxime Ceftriaxone
				Chloramphenicol[b]
GROUP U SUPPLEMENTAL FOR URINE ONLY	Tetracycline[a]			Sulfisoxazole
				Tetracycline[a]

[*] MIC testing only; disk diffusion test unreliable.
Abbreviations: **CoNS, coagulase-negative staphylococci;** MIC, minimal inhibitory concentration; UTI, urinary tract infection.

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Table 1A. (Continued)

"Warning": The following antimicrobial agents that are included in this document should not be routinely reported for bacteria isolated from CSF. These antimicrobial agents are not the drugs of choice and may not be effective for treating CSF infections caused by these organisms (ie, the bacteria included in Tables 2A through 2J):

- Agents administered by oral route only
- 1st- and 2nd-generation cephalosporins and cephamycins
- Clindamycin
- Macrolides
- Tetracyclines
- Fluoroquinolones

NOTE 1: For information about the selection of appropriate antimicrobial agents; explanation of test/report groups A, B, C, and U; and explanation of the listing of agents within boxes, including the meaning of "or" between agents, refer to the Instructions for Use of Tables that precede Table 1A.

NOTE 2: Information in boldface type is new or modified since the previous edition.

Footnotes

General

a. Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. However, some organisms that are intermediate or resistant to tetracycline may be susceptible to doxycycline, minocycline, or both.

b. Not routinely reported on organisms isolated from the urinary tract.

Enterobacteriaceae

c. **WARNING:** For *Salmonella* spp. and *Shigella* spp., aminoglycosides, first- and second-generation cephalosporins, and cephamycins may appear active *in vitro,* but are not effective clinically and should not be reported as susceptible.

When fecal isolates of *Salmonella* and *Shigella* spp. are tested, only ampicillin, a fluoroquinolone, and trimethoprim-sulfamethoxazole should be reported routinely. In addition, for extraintestinal isolates of *Salmonella* spp., a third-generation cephalosporin should be tested and reported, and chloramphenicol may be tested and reported, if requested. Susceptibility testing is indicated for typhoidal *Salmonella* (*S.* Typhi and *Salmonella* Paratyphi A–C) isolated from extraintestinal and intestinal sources. Routine susceptibility testing is not indicated for nontyphoidal *Salmonella* spp. isolated from intestinal sources. In contrast, susceptibility testing is indicated for all *Shigella* isolates.

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Table 1A. (Continued)

d. Cefotaxime or ceftriaxone should be tested and reported on isolates from CSF in place of cefazolin.

e. For testing and reporting of *E. coli* urinary tract isolates only.

Other Non-*Enterobacteriaceae*

f. Other non-*Enterobacteriaceae* include *Pseudomonas* spp. and other nonfastidious, glucose-nonfermenting, gram-negative bacilli, but exclude *P. aeruginosa, Acinetobacter* spp., *B. cepacia,* and *S. maltophilia,* because there are separate lists of suggested drugs to test and report for them.

Recommendations for testing and reporting of *Aeromonas hydrophila* complex, *Burkholderia mallei, Burkholderia pseudomallei,* and *Vibrio* species (including *V. cholerae*) are found in CLSI document M45.[1]

Staphylococcus spp.

g. ***Rx:*** Rifampin should not be used alone for antimicrobial therapy.

h. For *S. aureus* only, including methicillin-resistant *S. aureus* (MRSA).

i. Penicillin-susceptible staphylococci are also susceptible to other β-lactam agents with established clinical efficacy for staphylococcal infections. Penicillin-resistant staphylococci are resistant to penicillinase-labile penicillins. Oxacillin-resistant staphylococci are resistant to all currently available β-lactam antimicrobial agents, with the exception of the newer cephalosporins with anti-MRSA activity. Thus, susceptibility or resistance to a wide array of β-lactam antimicrobial agents may be deduced from testing only penicillin and either cefoxitin or oxacillin. Routine testing of other β-lactam agents, except those with anti-MRSA activity, is not advised.

j. Daptomycin should not be reported for isolates from the respiratory tract.

k. **If a penicillinase-stable penicillin is tested, oxacillin is the preferred agent, and results can be applied to the other penicillinase-stable penicillins (refer to Glossary I). Detection of oxacillin resistance in staphylococci is achieved by using specific methods as described in Tables 2C and 3E.**

l. For staphylococci that test susceptible, **gentamicin is** used only in combination with other active agents that test susceptible.

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Table 1A. (Continued)

Enterococcus spp.

m. **Warning:** For *Enterococcus* spp., cephalosporins, aminoglycosides (except for high-level resistance testing), clindamycin, and trimethoprim-sulfamethoxazole may appear active *in vitro,* but are not effective clinically and should not be reported as susceptible.

n. The results of ampicillin susceptibility tests should be used to predict the activity of amoxicillin. Ampicillin results may be used to predict susceptibility to amoxicillin-clavulanate, ampicillin-sulbactam, and piperacillin-tazobactam among non–β-lactamase-producing enterococci. Ampicillin susceptibility can be used to predict imipenem susceptibility, providing the species is confirmed to be *Enterococcus faecalis.*

o. Enterococci susceptible to penicillin are predictably susceptible to ampicillin, amoxicillin, ampicillin-sulbactam, amoxicillin-clavulanate, and piperacillin-tazobactam for non–β-lactamase-producing enterococci. However, enterococci susceptible to ampicillin cannot be assumed to be susceptible to penicillin. If penicillin results are needed, testing of penicillin is required. ***Rx:*** Combination therapy with ampicillin, penicillin, or vancomycin (for susceptible strains) plus an aminoglycoside is usually indicated for serious enterococcal infections, such as endocarditis, unless high-level resistance to both gentamicin and streptomycin is documented; such combinations are predicted to result in synergistic killing of the *Enterococcus.* **For strains with low-level penicillin or ampicillin resistance when combination therapy with a β-lactam is being considered, see additional testing and reporting information in Table 3I.[2]**

p. For testing and reporting of *E. faecalis* only.

q. For testing and reporting of *E. faecalis* urinary tract isolates only.

r. For testing and reporting of vancomycin-susceptible *E. faecalis* only.

NOTE: Information in boldface type is new or modified since the previous edition.

References for Table 1A

1 CLSI. *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria*. 3rd ed. CLSI guideline M45. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.

2 Murray BE, Arias CA, Nannini EC. Glycopeptides (vancomycin and teicoplanin), streptogramins (quinupristin-dalfopristin), lipopeptides (daptomycin), and lipoglycopeptides (telavancin). In: Bennett JE, Dolin R, Blaser MJ. *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*. 8th ed. Philadelphia, PA: Elsevier Saunders, 2015:377-400.

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Table 1B. Suggested Groupings of Antimicrobial Agents Approved by the US Food and Drug Administration for Clinical Use That Should Be Considered for Testing and Reporting on Fastidious Organisms by Microbiology Laboratories in the United States

	Haemophilus influenzae*[d] and *Haemophilus parainfluenzae	***Neisseria gonorrhoeae*[i]**	***Streptococcus pneumoniae*[j]**	***Streptococcus* spp. β-Hemolytic Group[p]**	***Streptococcus* spp. Viridans Group[p]**
GROUP A PRIMARY TEST AND REPORT	Ampicillin[d,f]	Ceftriaxone[†] Cefixime[†]	Erythromycin[a,c]	Clindamycin[c,o]	Ampicillin[m,*] Penicillin[m,*]
		Ciprofloxacin[†]	Penicillin[k] (oxacillin disk)	Erythromycin[a,c,o]	
		Tetracycline[b,†]	Trimethoprim-sulfamethoxazole	Penicillin[n,†] or ampicillin[n,†]	
GROUP B OPTIONAL PRIMARY TEST REPORT SELECTIVELY	Ampicillin-sulbactam		Cefepime[*] Cefotaxime[k,*] Ceftriaxone[k,*]	Cefepime or cefotaxime or ceftriaxone	Cefepime Cefotaxime Ceftriaxone
	Cefotaxime[d] or ceftazidime[d] or ceftriaxone[d]		Clindamycin[c]	Vancomycin	Vancomycin
	Ciprofloxacin or levofloxacin or moxifloxacin		Doxycycline		
			Levofloxacin[j] Moxifloxacin[j]		
	Meropenem[d]		Meropenem[k,*]		
			Tetracycline[b]		
			Vancomycin[k]		

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Table 1B. (Continued)

	Haemophilus influenzae[d] and *Haemophilus parainfluenzae*	*Neisseria gonorrhoeae*[i]	*Streptococcus pneumoniae*[j]	*Streptococcus* spp. β-Hemolytic Group[p]	*Streptococcus* spp. Viridans Group[p]
GROUP C SUPPLEMENTAL REPORT SELECTIVELY	Azithromycin[e] Clarithromycin[e]		Amoxicillin[*] Amoxicillin-clavulanate[*]	Ceftaroline	Ceftolozane-tazobactam
	Aztreonam		Cefuroxime[*]	Chloramphenicol[c]	Chloramphenicol[c]
	Amoxicillin-clavulanate[e]		Ceftaroline	Daptomycin[q,*]	Clindamycin[c]
	Cefaclor[e] Cefprozil[e]		Chloramphenicol[c]	Levofloxacin	Erythromycin[a,c]
	Cefdinir[e] or cefixime[e] or cefpodoxime[e]		Ertapenem[*] Imipenem[*]	Linezolid Tedizolid[r]	Linezolid Tedizolid[s]
				Dalbavancin[t,*]	**Dalbavancin[t,*]**
			Linezolid	Oritavancin[*]	Oritavancin[*]
	Ceftaroline[g]		Rifampin[l]	Telavancin[*]	Telavancin[*]
	Cefuroxime[e]				
	Chloramphenicol[c]				
	Ertapenem or imipenem				
	Rifampin[h]				
	Tetracycline[b]				
	Trimethoprim-sulfamethoxazole				

[*] MIC testing only; disk diffusion test unreliable.
[†] Routine testing is not necessary (see footnotes i and n).
Abbreviation: MIC, minimal inhibitory concentration.

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Table 1B. (Continued)

"Warning": The following antimicrobial agents that are included in this document should not be routinely reported for bacteria isolated from CSF. These antimicrobial agents are not the drugs of choice and may not be effective for treating CSF infections caused by these organisms (ie, the bacteria included in Tables 2A through 2J):

- Agents administered by oral route only
- 1st- and 2nd-generation cephalosporins and cephamycins
- Clindamycin
- Macrolides
- Tetracyclines
- Fluoroquinolones

NOTE: For information about the selection of appropriate antimicrobial agents; explanation of test/report groups A, B, C, and U; and explanation of the listing of agents within boxes, including the meaning of "or" between agents, refer to the Instructions for Use of Tables that precede Table 1A.

Footnotes

General

a. Susceptibility and resistance to azithromycin and clarithromycin can be predicted by testing erythromycin.

b. Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline.

c. Not routinely reported for organisms isolated from the urinary tract.

Haemophilus spp.

d. For isolates of *H. influenzae* from CSF, only results of testing with ampicillin, any of the third-generation cephalosporins listed, and meropenem are appropriate to report.

e. Amoxicillin-clavulanate, azithromycin, cefaclor, cefdinir, cefixime, cefpodoxime, cefprozil, cefuroxime, and clarithromycin are used as empiric therapy for respiratory tract infections due to *Haemophilus* spp. The results of susceptibility tests with these antimicrobial agents are often not necessary for managing individual patients.

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Table 1B. (Continued)

f. The results of ampicillin susceptibility tests should be used to predict the activity of amoxicillin. The majority of *H. influenzae* isolates that are resistant to ampicillin and amoxicillin produce a TEM-type β-lactamase. In most cases, a direct β-lactamase test can provide a rapid means of detecting ampicillin and amoxicillin resistance.

g. For *H. influenzae* only.

h. May be appropriate only for prophylaxis of case contacts. Refer to Table 2E.

Neisseria gonorrhoeae

i. Culture and susceptibility testing of *N. gonorrhoeae* should be considered in cases of treatment failure. Antimicrobial agents recommended for testing include, at a minimum, the agents listed in group A. The most current guidelines for treatment and testing are available from the Centers for Disease Control and Prevention at https://www.cdc.gov/std/gonorrhea/stdfact-gonorrhea.htm.

Streptococcus pneumoniae

j. *S. pneumoniae* isolates susceptible to levofloxacin are predictably susceptible to gemifloxacin and moxifloxacin. However, *S. pneumoniae* susceptible to gemifloxacin or moxifloxacin cannot be assumed to be susceptible to levofloxacin.

k. Penicillin and cefotaxime, ceftriaxone, or meropenem should be tested by a reliable MIC method (such as that described in M07[1]), and reported routinely with CSF isolates of *S. pneumoniae*. Such isolates can also be tested against vancomycin using the MIC or disk diffusion method. With isolates from other sites, the oxacillin disk test may be used. If the oxacillin zone size is ≤ 19 mm, penicillin, cefotaxime, ceftriaxone, or meropenem MICs should be determined.

l. ***Rx:*** Rifampin should not be used alone for antimicrobial therapy.

Streptococcus spp.

m. ***Rx:*** Penicillin- or ampicillin-intermediate isolates may necessitate combined therapy with an aminoglycoside for bactericidal action.

n. Penicillin and ampicillin are drugs of choice for treating β-hemolytic streptococcal infections. Susceptibility testing of penicillins and other β-lactams approved by the US Food and Drug Administration for treating β-hemolytic streptococcal infections does not need to be performed routinely, because nonsusceptible isolates (ie, penicillin MICs > 0.12 and ampicillin MICs > 0.25 μg/mL) are extremely rare in any β-hemolytic streptococci and have not been reported for *Streptococcus pyogenes.* If testing is performed, any β-hemolytic streptococcal isolate found to be nonsusceptible should be re-identified, retested, and, if confirmed, submitted to a public health laboratory. (See Appendix A for additional instructions.)

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Table 1B. (Continued)

o. ***Rx:*** Recommendations for intrapartum prophylaxis for group B streptococci are penicillin or ampicillin. Although cefazolin is recommended for penicillin-allergic women at low risk for anaphylaxis, those at high risk for anaphylaxis may receive clindamycin. Group B streptococci are susceptible to ampicillin, penicillin, and cefazolin, but may be resistant to erythromycin and clindamycin. When group B *Streptococcus* is isolated from a pregnant woman with severe penicillin allergy (high risk for anaphylaxis), erythromycin and clindamycin (including inducible clindamycin resistance) should be tested, and only clindamycin should be reported. See Table 3**G**.

p. For this table, the β-hemolytic group includes the large colony–forming pyogenic strains of streptococci with group A (*S. pyogenes*), C, or G antigens and strains with group B (*S. agalactiae*) antigen. Small colony–forming β-hemolytic strains with group A, C, F, or G antigens (*Streptococcus anginosus* group, previously termed *"Streptococcus milleri"*) are considered part of the viridans group, and breakpoints for the viridans group should be used.

q. Daptomycin should not be reported for isolates from the respiratory tract.

r. For reporting against *S. pyogenes* and *Streptococcus agalactiae* only.

s. For reporting against *S. anginosus* group (includes *S. anginosus, Streptococcus intermedius,* and *Streptococcus constellatus*) only.

t. For reporting against *S. pyogenes*, *S. agalactiae*, *Streptococcus dysgalactiae*, and *S. anginosus* group.

NOTE: Information in boldface type is new or modified since the previous edition.

Reference for Table 1B

1 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

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Table 1C. Suggested Groupings of Antimicrobial Agents Approved by the US Food and Drug Administration for Clinical Use That Should Be Considered for Testing and Reporting on Anaerobic Organisms by Microbiology Laboratories in the United States

	***Bacteroides fragilis* Group and Other Gram-Negative Anaerobes**	**Gram-Positive Anaerobes[a]**
GROUP A PRIMARY TEST AND REPORT	Amoxicillin-clavulanate Ampicillin-sulbactam Piperacillin-tazobactam	Ampicillin[b] Penicillin[b]
		Amoxicillin-clavulanate Ampicillin-sulbactam Piperacillin-tazobactam
	Clindamycin	Clindamycin
	Doripenem Ertapenem Imipenem Meropenem	Doripenem Ertapenem Imipenem Meropenem
	Metronidazole	Metronidazole
GROUP C SUPPLEMENTAL REPORT SELECTIVELY	Penicillin[b] Ampicillin[b]	
	Cefotetan Cefoxitin	Cefotetan Cefoxitin
	Ceftizoxime Ceftriaxone	Ceftizoxime Ceftriaxone
	Chloramphenicol	
	Moxifloxacin	Moxifloxacin
		Tetracycline

NOTE 1: For information about the selection of appropriate antimicrobial agents; explanation of test/report groups A and C; and explanation of the listing of agents within boxes, refer to the Instructions for Use of Tables that precede Table 1A.

NOTE 2: Most anaerobic infections are polymicrobial, including both β-lactamase-positive and β-lactamase-negative strains. Testing may not be necessary for isolates associated with polymicrobial anaerobic infections. However, if susceptibility testing is requested, only the organism most likely to be resistant (eg, *B. fragilis* group) should be tested and results reported (see Appendix D).

NOTE 3: Specific *Clostridium* spp. (eg, *Clostridium septicum, Clostridium sordellii*) may be the singular cause of infection and are typically susceptible to penicillin and ampicillin. Penicillin and clindamycin resistance has been reported in *Clostridium perfringens*. Agents in group A of Table 1C should be tested and reported for *Clostridium* spp.

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Table 1C. (Continued)

Footnotes

a. Many non-spore-forming, gram-positive anaerobic rods are resistant to metronidazole (see Appendix D).

b. If β-lactamase positive, report as resistant to penicillin and ampicillin. Be aware that β-lactamase-negative isolates may be resistant to penicillin and ampicillin by other mechanisms.

NOTE: Information in boldface type is new or modified since the previous edition.

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Table 2A. Zone Diameter and MIC Breakpoints for *Enterobacteriaceae*

Testing Conditions

Medium: Disk diffusion: MHA
Broth dilution: CAMHB
Agar dilution: MHA

Inoculum: **Broth culture** method or colony suspension, equivalent to a 0.5 McFarland standard

Incubation: 35°C ± 2°C; ambient air
Disk diffusion: 16–18 hours
Dilution methods: 16–20 hours

Routine QC Recommendations (see Tables 4A-1 and 5A-1 for acceptable QC ranges)

Escherichia coli ATCC®* 25922
Pseudomonas aeruginosa ATCC® 27853 (for carbapenems)

Refer to Tables 4A-2 and 5A-2 to select strains for routine QC of β-lactam combination agents.

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

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

Refer to Tables 3A, 3B, and 3C for additional testing, reporting, and QC for *Enterobacteriaceae*.

General Comments

(1) For disk diffusion, test a maximum of 12 disks on a 150-mm plate and no more than 6 disks on a 100-mm plate; disks should be placed no less than 24 mm apart, center to center (see M02,[1] Subchapter 3.6). Each zone diameter should be clearly measurable; overlapping zones prevent accurate measurement. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Hold the Petri plate a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. Strains of *Proteus* spp. may swarm into areas of inhibited growth around certain antimicrobial agents. With *Proteus* spp., ignore the thin veil of swarming growth in an otherwise obvious zone of growth inhibition. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.

(2) When fecal isolates of *Salmonella* and *Shigella* spp. are tested, only ampicillin, a fluoroquinolone, and trimethoprim-sulfamethoxazole should be reported routinely. In addition, for extraintestinal isolates of *Salmonella* spp., a 3rd-generation cephalosporin should be tested and reported, and chloramphenicol may be tested and reported if requested. Susceptibility testing is indicated for typhoidal *Salmonella* (*S.* Typhi and *S.* Paratyphi A–C) isolated from extraintestinal and intestinal sources. Routine susceptibility testing is not indicated for nontyphoidal *Salmonella* spp. isolated from intestinal sources. In contrast, susceptibility testing is indicated for all *Shigella* isolates.

(3) The dosage regimens shown in the comments column below are those needed to achieve plasma drug exposures (in adults with normal renal and hepatic functions) on which breakpoints were based. When implementing new breakpoints, it is strongly recommended that laboratories share this information with infectious diseases practitioners, pharmacists, pharmacy and therapeutics committees, infection control committees, **and the antimicrobial stewardship team.**

NOTE: Information in boldface type is new or modified since the previous edition.

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Table 2A. *Enterobacteriaceae* (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
PENICILLINS											
A	Ampicillin	10 µg	≥17	–	14–16	≤13	≤8	–	16	≥32	**(4)** Results of ampicillin testing can be used to predict results for amoxicillin. See general comment (2).
O	Piperacillin	100 µg	≥21	–	18–20	≤17	≤16	–	32–64	≥128	
O	Mecillinam	10 µg	≥15	–	12–14	≤11	≤8	–	16	≥32	**(5)** For testing and reporting of *E. coli* urinary tract isolates only.
β-LACTAM COMBINATION AGENTS											
B	Amoxicillin-clavulanate	20/10 µg	≥18	–	14–17	≤13	≤8/4	–	16/8	≥32/16	
B	Ampicillin-sulbactam	10/10 µg	≥15	–	12–14	≤11	≤8/4	–	16/8	≥32/16	
B	Ceftolozane-tazobactam	**30/10** µg	**≥21**	–	**18–20**	**≤17**	≤2/4	–	4/4	≥8/4	**(6)** Breakpoints are based on a dosage regimen of 1.5 g every 8 h.
B	**Ceftazidime-avibactam**	**30/20 µg**	**≥21**	**–**	**–**	**≤20**	**≤8/4**	**–**	**–**	**≥16/4**	**(7) Breakpoints are based on a dosage regimen of 2.5 g (2 g ceftazidime + 0.5 g avibactam) every 8 h over 2 days.**
B	Piperacillin-tazobactam	100/10 µg	≥21	–	18–20	≤17	≤16/4	–	32/4–64/4	≥128/4	
O	Ticarcillin-clavulanate	75/10 µg	≥20	–	15–19	≤14	≤16/2	–	32/2–64/2	≥128/2	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.) **(8) WARNING:** For *Salmonella* spp. and *Shigella* spp., 1st- and 2nd-generation cephalosporins and cephamycins may appear active *in vitro,* but are not effective clinically and should not be reported as susceptible. **(9)** Following evaluation of PK-PD properties, limited clinical data, and MIC distributions, revised breakpoints for cephalosporins (cefazolin, cefotaxime, ceftazidime, ceftizoxime, and ceftriaxone) and aztreonam were first published in January 2010 (M100-S20) and are listed in this table. Cefuroxime (parenteral) was also evaluated; however, no change in breakpoints was necessary for the dosage indicated below. When using the current breakpoints, routine ESBL testing is no longer necessary before reporting results (ie, it is no longer necessary to edit results for cephalosporins, aztreonam, or penicillins from susceptible to resistant). However, ESBL testing may still be useful for epidemiological or infection control purposes. For laboratories that have not implemented the current breakpoints, ESBL testing should be performed as described in Table 3A. Note that breakpoints for drugs with limited availability in many countries (eg, moxalactam, cefonicid, cefamandole, and cefoperazone) were not evaluated. If considering use of these drugs for *E. coli, Klebsiella* spp., or *Proteus* spp., ESBL testing should be performed (see Table 3A). If isolates test ESBL positive, the results for moxalactam, cefonicid, cefamandole, and cefoperazone should be reported as resistant. **(10)** *Enterobacter, Citrobacter,* and *Serratia* may develop resistance during prolonged therapy with 3rd-generation cephalosporins as a result of derepression of AmpC β-lactamase. Therefore, isolates that are initially susceptible may become resistant within 3 to 4 days after initiation of therapy. Testing repeat isolates may be warranted.											
A	Cefazolin	30 µg	≥23	–	20–22	≤19	≤2	–	4	≥8	**(11)** Breakpoints when cefazolin is used for therapy of infections other than uncomplicated UTIs due to *E. coli, K. pneumoniae,* and *P. mirabilis*. Breakpoints are based on a dosage regimen of 2 g every 8 h. See comment (9).

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Table 2A. *Enterobacteriaceae* (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.) (Continued)											
U	Cefazolin	30 µg	≥15	–	–	≤14	≤16	–	–	≥32	**(12)** Breakpoints when cefazolin is used for therapy of uncomplicated UTIs due to *E. coli, K. pneumoniae,* and *P. mirabilis*. Breakpoints are based on a dosage regimen of 1 g every 12 h. See additional information in CEPHEMS (ORAL).
C	Ceftaroline	30 µg	≥23	–	20–22	≤19	≤0.5	–	1	≥2	**(13)** Breakpoints are based on a dosage regimen of 600 mg every 12 h.
B	Cefepime	30 µg	≥25	19–24	–	≤18	≤2	4–8	–	≥16	**(14)** The breakpoint for susceptible is based on a dosage regimen of 1 g every 12 h. The breakpoint for SDD is based on dosage regimens that result in higher cefepime exposure, either higher doses or more frequent doses or both, up to approved maximum dosage regimens. See Appendix E for more information about breakpoints and dosage regimens. Also see the definition of SDD in the Instructions for Use of Tables section.
B B	Cefotaxime or ceftriaxone	30 µg 30 µg	≥26 ≥23	–	23–25 20–22	≤22 ≤19	≤1 ≤1	–	2 2	≥4 ≥4	**(15)** Breakpoints are based on a dosage regimen of 1 g every 24 h for ceftriaxone and 1 g every 8 h for cefotaxime. See comment (9).
B	Cefotetan	30 µg	≥16	–	13–15	≤12	≤16	–	32	≥64	
B	Cefoxitin	30 µg	≥18	–	15–17	≤14	≤8	–	16	≥32	**(16)** Breakpoints are based on a dosage regimen of at least 8 g per day (eg, 2 g every 6 h).
B	Cefuroxime (parenteral)	30 µg	≥18	–	15–17	≤14	≤8	–	16	≥32	**(17)** Breakpoints are based on a dosage regimen of 1.5 g every 8 h. See comment (9).
C	Ceftazidime	30 µg	≥21	–	18–20	≤17	≤4	–	8	≥16	**(18)** Breakpoints are based on a dosage regimen of 1 g every 8 h. See comment (9).
O	Cefamandole	30 µg	≥18	–	15–17	≤14	≤8	–	16	≥32	See comment (9).
O	Cefmetazole	30 µg	≥16	–	13–15	≤12	≤16	–	32	≥64	**(19)** Insufficient new data exist to reevaluate breakpoints listed here.

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Table 2A. *Enterobacteriaceae* (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.) (Continued)											
O	Cefonicid	30 µg	≥18	–	15–17	≤14	≤8	–	16	≥32	See comment (9).
O	Cefoperazone	75 µg	≥21	–	16–20	≤15	≤16	–	32	≥64	See comment (9).
O	Ceftizoxime	30 µg	≥25	–	22–24	≤21	≤1	–	2	≥4	**(20)** Breakpoints are based on a dosage regimen of 1 g every 12 h. See comment (9).
O	Moxalactam	30 µg	≥23	–	15–22	≤14	≤8	–	16–32	≥64	See comment (9).
CEPHEMS (ORAL)											
B	Cefuroxime	30 µg	≥23		15–22	≤14	≤4		8–16	≥32	See comment (21).
U	Cefazolin (surrogate test for oral cephalosporins and uncomplicated UTIs)	30 µg	≥15	–	–	≤14	≤16	–	–	≥32	**(21)** Breakpoints are for cefazolin when cefazolin results are used to predict results for the oral agents cefaclor, cefdinir, cefpodoxime, cefprozil, cefuroxime, cephalexin, and loracarbef when used for therapy of uncomplicated UTIs due to *E. coli, K. pneumoniae,* and *P. mirabilis*. **Cefazolin as a surrogate may overcall resistance to cefdinir, cefpodoxime, and cefuroxime. If cefazolin tests resistant, test these drugs individually if needed for therapy**.
O	Loracarbef	30 µg	≥18	–	15–17	≤14	≤8	–	16	≥32	**(22)** Do not test *Citrobacter, Providencia,* or *Enterobacter* spp. with cefdinir or loracarbef by disk diffusion because false-susceptible results have been reported. See comment (21).
O	Cefaclor	30 µg	≥18	–	15–17	≤14	≤8	–	16	≥32	See comment (21).
O	Cefdinir	5 µg	≥20	–	17–19	≤16	≤1	–	2	≥4	See comments (21) and (22).
O	Cefixime	5 µg	≥19	–	16–18	≤15	≤1	–	2	≥4	**(23)** Do not test *Morganella* spp. with cefixime, cefpodoxime, or cefetamet by disk diffusion.
O	Cefpodoxime	10 µg	≥21	–	18–20	≤17	≤2	–	4	≥8	See comments (21) and (23).

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Table 2A. *Enterobacteriaceae* (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
CEPHEMS (ORAL) (Continued)											
O	Cefprozil	30 µg	≥18	–	15–17	≤14	≤8	–	16	≥32	**(24)** Do not test *Providencia* spp. with cefprozil by disk diffusion because false-susceptible results have been reported. See comment (21).
Inv.	Cefetamet	10 µg	≥18	–	15–17	≤14	≤4	–	8	≥16	See comment (23).
Inv.	Ceftibuten	30 µg	≥21	–	18–20	≤17	≤8	–	16	≥32	**(25)** For testing and reporting of urinary tract isolates only.
MONOBACTAMS											
C	Aztreonam	30 µg	≥21	–	18–20	≤17	≤4	–	8	≥16	**(26)** Breakpoints are based on a dosage regimen of 1 g every 8 h. See comment (9).
CARBAPENEMS **(27)** Following evaluation of PK-PD properties, limited clinical data, and MIC distributions that include recently described carbapenemase-producing strains, revised breakpoints for carbapenems were first published in June 2010 (M100-S20-U) and are listed below. Because of limited treatment options for infections caused by organisms with carbapenem MICs or zone diameters in the intermediate range, clinicians may wish to design carbapenem dosage regimens that use maximum recommended doses and possibly prolonged intravenous infusion regimens, as has been reported in the literature.[2-5] Consultation with an infectious diseases practitioner is recommended for isolates for which the carbapenem MICs or zone diameter results from disk diffusion testing are in the intermediate or resistant ranges. Laboratories using *Enterobacteriaceae* MIC breakpoints for carbapenems described in M100-S20 (January 2010) should perform the CarbaNP test, mCIM, **eCIM**, and/or a molecular assay when isolates of *Enterobacteriaceae* are suspicious for carbapenemase production based on imipenem or meropenem MICs of 2–4 µg/mL or ertapenem MIC of 2 µg/mL (refer to Tables 3B and 3C). After implementation of the current breakpoints, these additional tests do not need to be performed other than for epidemiological or infection control purposes. The following information is provided as background on carbapenemases in *Enterobacteriaceae* that are largely responsible for MICs and zone diameters in the intermediate and resistant ranges, and thus the rationale for setting revised carbapenem breakpoints: • The clinical effectiveness of carbapenem treatment of infections produced by isolates for which the carbapenem MIC or disk diffusion test results are within the intermediate range is uncertain due to lack of controlled clinical studies. • Imipenem MICs for *Proteus* spp., *Providencia* spp., and *Morganella morganii* tend to be higher (eg, MICs in the intermediate or resistant range) than meropenem or doripenem MICs. These isolates may have elevated imipenem MICs by mechanisms other than production of carbapenemases.										
B	Doripenem	10 µg	≥23	–	20–22	≤19	≤1	–	2	≥4	**(28)** Breakpoints are based on a dosage regimen of 500 mg every 8 h.
B	Ertapenem	10 µg	≥22	–	19–21	≤18	≤0.5	–	1	≥2	**(29)** Breakpoints are based on a dosage regimen of 1 g every 24 h.
B	Imipenem	10 µg	≥23	–	20–22	≤19	≤1	–	2	≥4	**(30)** Breakpoints are based on a dosage regimen of 500 mg every 6 h or 1 g every 8 h.
B	Meropenem	10 µg	≥23	–	20–22	≤19	≤1	–	2	≥4	**(31)** Breakpoints are based on a dosage regimen of 1 g every 8 h.

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Table 2A. *Enterobacteriaceae* (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
AMINOGLYCOSIDES **(32) WARNING:** For *Salmonella* spp. and *Shigella* spp., aminoglycosides may appear active *in vitro* but are not effective clinically and should not be reported as susceptible.											
A	Gentamicin	10 µg	≥15	–	13–14	≤12	≤4	–	8	≥16	
A	Tobramycin	10 µg	≥15	–	13–14	≤12	≤4	–	8	≥16	
B	Amikacin	30 µg	≥17	–	15–16	≤14	≤16	–	32	≥64	
O	Kanamycin	30 µg	≥18	–	14–17	≤13	≤16	–	32	≥64	
O	Netilmicin	30 µg	≥15	–	13–14	≤12	≤8	–	16	≥32	
O	Streptomycin	10 µg	≥15	–	12–14	≤11	–	–	–	–	**(33)** There are no MIC breakpoints.
MACROLIDES											
Inv.	Azithromycin	15 µg	≥13	–	–	≤12	≤16	–	–	≥32	**(34)** *S.* Typhi only: breakpoints are based on MIC distribution data and limited clinical data. For *S. flexneri* and *S. sonnei,* see **Appendix G, Table G1**.
TETRACYCLINES **(35)** Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. However, some organisms that are intermediate or resistant to tetracycline may be susceptible to doxycycline, minocycline, or both.											
C	Tetracycline	30 µg	≥15	–	12–14	≤11	≤4	–	8	≥16	
O	Doxycycline	30 µg	≥14	–	11–13	≤10	≤4	–	8	≥16	
O	Minocycline	30 µg	≥16	–	13–15	≤12	≤4	–	8	≥16	
QUINOLONES AND FLUOROQUINOLONES for *Enterobacteriaceae* except *Salmonella* spp. (Please refer to Glossary I.)											
B	Ciprofloxacin	5 µg	≥21	–	16–20	≤15	≤1	–	2	≥4	
B	Levofloxacin	5 µg	≥17		14–16	≤13	≤2	–	4	≥8	
O	Cinoxacin	100 µg	≥19	–	15–18	≤14	≤16	–	32	≥64	See comment (25).
O	Enoxacin	10 µg	≥18	–	15–17	≤14	≤2	–	4	≥8	See comment (25).
O	Gatifloxacin	5 µg	≥18	–	15–17	≤14	≤2	–	4	≥8	
O	Gemifloxacin	5 µg	≥20	–	16–19	≤15	≤0.25	–	0.5	≥1	**(36) For testing and reporting of *K. pneumoniae* only.**
O	Grepafloxacin	5 µg	≥18	–	15–17	≤14	≤1	–	2	≥4	
O	Lomefloxacin	10 µg	≥22	–	19–21	≤18	≤2	–	4	≥8	
O	Nalidixic acid	30 µg	≥19	–	14–18	≤13	≤16	–	–	≥32	See comment (25).
O	Norfloxacin	10 µg	≥17	–	13–16	≤12	≤4	–	8	≥16	See comment (25).
O	Ofloxacin	5 µg	≥16	–	13–15	≤12	≤2	–	4	≥8	
Inv.	Fleroxacin	5 µg	≥19	–	16–18	≤15	≤2	–	4	≥8	

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Table 2A. E*nterobacteriaceae* (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
QUINOLONES AND FLUOROQUINOLONES for *Salmonella* spp. (Please refer to Glossary I.) **(37)** For testing and reporting of *Salmonella* spp. (including *S.* Typhi and *S.* Paratyphi A–C). Routine susceptibility testing is not indicated for nontyphoidal *Salmonella* spp. isolated from intestinal sources. **(38)** The preferred test for assessing fluoroquinolone susceptibility or resistance in *Salmonella* spp. is a ciprofloxacin MIC test. A levofloxacin or ofloxacin MIC test can be performed if either agent, respectively, is the fluoroquinolone of choice in a specific facility. If a ciprofloxacin, levofloxacin, or ofloxacin MIC or ciprofloxacin disk diffusion test cannot be done, pefloxacin disk diffusion may be used as surrogate test to predict ciprofloxacin susceptibility. **(39)** No single test detects resistance resulting from all possible fluoroquinolone resistance mechanisms that have been identified in *Salmonella* spp.											
B B	Ciprofloxacin Levofloxacin	5 µg –	≥31 –	– –	21–30 –	≤20 –	≤0.06 ≤0.12	– –	0.12–0.5 0.25–1	≥1 ≥2	**(40)** Isolates of *Salmonella* spp. that test not susceptible to ciprofloxacin, levofloxacin, ofloxacin, or pefloxacin may be associated with clinical failure or delayed response in fluoroquinolone-treated patients with salmonellosis.
O	Ofloxacin	–	–	–	–	–	≤0.12	–	0.25–1	≥2	
Inv.	Pefloxacin (surrogate test for ciprofloxacin)	5 µg	≥24	–	–	≤23	–	–	–	–	**(41)** Report results as ciprofloxacin susceptible or resistant based on the pefloxacin test result. Pefloxacin will not detect resistance in *Salmonella* spp. due to *aac(6')-Ib-cr*. Pefloxacin disks are not available in the United States. See comment (39).
FOLATE PATHWAY ANTAGONISTS											
B	Trimethoprim-sulfamethoxazole	1.25/23.75 µg	≥16	–	11–15	≤10	≤2/38	–	–	≥4/76	See general comment (2).
U	Sulfonamides	250 or 300 µg	≥17	–	13–16	≤12	≤256	–	–	≥512	**(42)** Sulfisoxazole can be used to represent any of the currently available sulfonamide preparations.
U	Trimethoprim	5 µg	≥16	–	11–15	≤10	≤8	–	–	≥16	
PHENICOLS											
C	Chloramphenicol	30 µg	≥18	–	13–17	≤12	≤8	–	16	≥32	**(43)** Not routinely reported on isolates from the urinary tract.

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Table 2A. *Enterobacteriaceae* (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
FOSFOMYCINS											
U	Fosfomycin	200 µg	≥16	–	13–15	≤12	≤64	–	128	≥256	**(44)** For testing and reporting of *E. coli* urinary tract isolates only. **(45)** The 200-µg fosfomycin disk contains 50 µg of glucose-6-phosphate. **(46)** The only approved MIC method for testing is agar dilution using agar media supplemented with 25 µg/mL of glucose-6-phosphate. Broth dilution MIC testing should not be performed.
NITROFURANS											
U	Nitrofurantoin	300 µg	≥17	–	15–16	≤14	≤32	–	64	≥128	

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; **eCIM, EDTA-modified carbapenem inactivation method;** ESBL, extended-spectrum β-lactamase; I, intermediate; mCIM, modified carbapenem inactivation method; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; PK-PD, pharmacokinetic-pharmacodynamic; QC, quality control; R, resistant; S, susceptible; SDD, susceptible-dose dependent; UTI, urinary tract infection.

References for Table 2A

1 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

2 Perrott J, Mabasa VH, Ensom MH. Comparing outcomes of meropenem administration strategies based on pharmacokinetic and pharmacodynamic principles: a qualitative systematic review. *Ann Pharmacother*. 2010;44(3):557-564.

3 Cirillo I, Vaccaro N, Turner K, Solanki B, Natarajan J, Redman R. Pharmacokinetics, safety, and tolerability of doripenem after 0.5-, 1-, and 4-hour infusions in healthy volunteers. *J Clin Pharmacol*. 2009;49(7):798-806.

4 Sakka SG, Glauner AK, Bulitta JB, et al. Population pharmacokinetics and pharmacodynamics of continuous versus short-term infusion of imipenem-cilastatin in critically ill patients in a randomized, controlled trial. *Antimicrob Agents Chemother*. 2007;51(9):3304-3310.

5 Peleg AY, Hooper DC. Hospital-acquired infections due to gram-negative bacteria. *N Engl J Med*. 2010;362(19):1804-1813.

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Table 2B-1. Zone Diameter and MIC Breakpoints for *Pseudomonas aeruginosa*

Testing Conditions

Medium: Disk diffusion: MHA
Broth dilution: CAMHB
Agar dilution: MHA

Inoculum: **Broth culture** method or colony suspension, equivalent to a 0.5 McFarland standard

Incubation: 35°C ± 2°C; ambient air
Disk diffusion: 16–18 hours
Dilution methods: 16–20 hours

Routine QC Recommendations (see Tables 4A-1 and 5A-1 for acceptable QC ranges)

Pseudomonas aeruginosa ATCC®* 27853

Refer to Tables 4A-2 and 5A-2 to select strains for routine QC of β-lactam combination agents.

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

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

General Comments

(1) For disk diffusion, test a maximum of 12 disks on a 150-mm plate and no more than 6 disks on a 100-mm plate; disks should be placed no less than 24 mm apart, center to center (see M02,[1] Subchapter 3.6). Each zone diameter should be clearly measurable; overlapping zones prevent accurate measurement. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Hold the Petri plate a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth.

(2) The susceptibility of *P. aeruginosa* isolated from patients with cystic fibrosis can be reliably determined by disk diffusion or dilution methods but may need extended incubation for up to 24 hours before reporting as susceptible.

(3) *P. aeruginosa* may develop resistance during prolonged therapy with all antimicrobial agents. Therefore, isolates that are initially susceptible may become resistant within 3 to 4 days after initiation of therapy. Testing of repeat isolates may be warranted.

(4) The dosage regimens shown in the comments column below are those necessary to achieve plasma drug exposures (in adults with normal renal and hepatic functions) on which breakpoints were derived. When implementing new breakpoints, it is strongly recommended that laboratories share this information with infectious diseases practitioners, pharmacists, pharmacy and therapeutics committees, infection control committees, **and the antimicrobial stewardship team**.

NOTE: Information in boldface type is new or modified since the previous edition.

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Table 2B-1. *Pseudomonas aeruginosa* (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
PENICILLINS									
O	Piperacillin	100 µg	≥21	15–20	≤14	≤16	32–64	≥128	**(5)** Breakpoints for piperacillin (alone or with tazobactam) are based on a piperacillin dosage regimen of at least 3 g every 6 h.
β-LACTAM COMBINATION AGENTS									
A	Piperacillin-tazobactam	100/10 µg	≥21	15–20	≤14	≤16/4	32/4–64/4	≥128/4	**(6)** Breakpoints for piperacillin (alone or with tazobactam) are based on a piperacillin dosage regimen of at least 3 g every 6 h.
B	**Ceftazidime-avibactam**	**30/20 µg**	**≥21**	**–**	**≤20**	**≤8/4**	**–**	**≥16/4**	**(7) Breakpoints are based on a dosage regimen of 2.5 g (2 g ceftazidime + 0.5 g avibactam) every 8 h over 2 days**
B	Ceftolozane-tazobactam	30/10 µg	≥21	17–20	≤16	≤4/4	8/4	≥16/4	**(8)** Breakpoints are based on a dosage regimen of 1.5 g every 8 h.
O	Ticarcillin-clavulanate	75/10 µg	≥24	16–23	≤15	≤16/2	32/2–64/2	≥128/2	**(9)** Breakpoints for ticarcillin (alone or with clavulanate) are based on a ticarcillin dosage regimen of at least 3 g every 6 h.
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)									
A	Ceftazidime	30 µg	≥18	15–17	≤14	≤8	16	≥32	**(10)** Breakpoints are based on a dosage regimen of 1 g every 6 h or 2 g every 8 h.
B	Cefepime	30 µg	≥18	15–17	≤14	≤8	16	≥32	**(11)** Breakpoints are based on a dosage regimen of 1 g every 8 h or 2 g every 12 h.
MONOBACTAMS									
B	Aztreonam	30 µg	≥22	16–21	≤15	≤8	16	≥32	**(12)** Breakpoints are based on a dosage regimen of 1 g every 6 h or 2 g every 8 h.
CARBAPENEMS									
B	Doripenem	10 µg	≥19	16–18	≤15	≤2	4	≥8	**(13)** Breakpoints for doripenem are based on a dosage regimen of 500 mg every 8 h.
B	Imipenem	10 µg	≥19	16–18	≤15	≤2	4	≥8	**(14)** Breakpoints for imipenem are based on a dosage regimen of 1 g every 8 h or 500 mg every 6 h.
B	Meropenem	10 µg	≥19	16–18	≤15	≤2	4	≥8	**(15)** Breakpoints for meropenem are based on a dosage regimen of 1 g every 8 h.

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Table 2B-1. *Pseudomonas aeruginosa* (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
LIPOPEPTIDES									
O	Colistin	–	–	–	–	≤2	–	≥4	**(16)** Colistin (methanesulfonate) should generally be administered with a loading dose and at the maximum recommended doses, in combination with other agents. **(17) The only approved MIC method for testing is broth microdilution. Disk diffusion and gradient diffusion methods should not be performed.**
O	Polymyxin B	–	–	–	–	≤2	4	≥8	
AMINOGLYCOSIDES									
A	Gentamicin	10 µg	≥15	13–14	≤12	≤4	8	≥16	
A	Tobramycin	10 µg	≥15	13–14	≤12	≤4	8	≥16	
B	Amikacin	30 µg	≥17	15–16	≤14	≤16	32	≥64	
O	Netilmicin	30 µg	≥15	13–14	≤12	≤8	16	≥32	
FLUOROQUINOLONES									
B	Ciprofloxacin	5 µg	≥21	16–20	≤15	≤ 1	2	≥4	
B	Levofloxacin	5 µg	≥17	14–16	≤13	≤ 2	4	≥8	
O	Norfloxacin	10 µg	≥17	13–16	≤12	≤4	8	≥16	**(18)** For testing and reporting of urinary tract isolates only.
O	Lomefloxacin	10 µg	≥22	19–21	≤18	≤ 2	4	≥8	See comment (18).
O	Ofloxacin	5 µg	≥16	13–15	≤12	≤ 2	4	≥8	
O	Gatifloxacin	5 µg	≥18	15–17	≤14	≤ 2	4	≥8	

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; I, intermediate; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; R, resistant; S, susceptible.

Reference for Table 2B-1

[1] CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

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Table 2B-2. Zone Diameter and MIC Breakpoints for *Acinetobacter* spp.

Testing Conditions

Medium: Disk diffusion: MHA
Broth dilution: CAMHB
Agar dilution: MHA

Inoculum: **Broth culture** method or colony suspension, equivalent to a 0.5 McFarland standard

Incubation: 35°C±2°C; ambient air; 20–24 hours, all methods

Routine QC Recommendations (see Tables 4A-1 and 5A-1 for acceptable QC ranges)

Escherichia coli ATCC®* 25922 (for tetracyclines and trimethoprim-sulfamethoxazole)
Pseudomonas aeruginosa ATCC® 27853

Refer to Tables 4A-2 and 5A-2 to select strains for routine QC of β-lactam combination agents.

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

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

General Comment

(1) For disk diffusion, test a maximum of 12 disks on a 150-mm plate and no more than 6 disks on a 100-mm plate; disks should be placed no less than 24 mm apart, center to center (see M02,[1] Subchapter 3.6). Each zone diameter should be clearly measurable; overlapping zones prevent accurate measurement. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Hold the Petri plate a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.

NOTE: Information in boldface type is new or modified since the previous edition.

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
PENICILLINS									
O	Piperacillin	100 µg	≥21	18–20	≤17	≤16	32–64	≥128	
β-LACTAM COMBINATION AGENTS									
A	Ampicillin-sulbactam	10/10 µg	≥15	12–14	≤11	≤8/4	16/8	≥32/16	
B	Piperacillin-tazobactam	100/10 µg	≥21	18–20	≤17	≤16/4	32/4–64/4	≥128/4	
O	Ticarcillin-clavulanate	75/10 µg	≥20	15–19	≤14	≤16/2	32/2–64/2	≥128/2	

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Table 2B-2. *Acinetobacter* spp. (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)									
A	Ceftazidime	30 µg	≥18	15–17	≤14	≤8	16	≥32	
B	Cefepime	30 µg	≥18	15–17	≤14	≤8	16	≥32	
B	Cefotaxime	30 µg	≥23	15–22	≤14	≤8	16–32	≥64	
B	Ceftriaxone	30 µg	≥21	14–20	≤13	≤8	16–32	≥64	
CARBAPENEMS									
A	Doripenem	10 µg	≥18	15–17	≤14	≤2	4	≥8	**(2)** Breakpoints for doripenem are based on a dosage regimen of 500 mg every 8 h.
A	Imipenem	10 µg	≥22	19–21	≤18	≤2	4	≥8	**(3)** Breakpoints for imipenem are based on a dosage regimen of 500 mg every 6 h.
A	Meropenem	10 µg	≥18	15–17	≤14	≤2	4	≥8	**(4)** Breakpoints for meropenem are based on a dosage regimen of 1 g every 8 h or 500 mg every 6 h.
LIPOPEPTIDES									
O	Colistin		–	–	–	≤2	–	≥4	**(5)** Colistin (methanesulfonate) should generally be given with a loading dose and at maximum recommended doses and used in combination with other agents. **(6)** Applies to *A. baumannii* complex only. **(7) The only approved MIC method for testing is broth microdilution. Disk diffusion and gradient diffusion methods should not be performed.**
O	Polymyxin B	–	–	–	–	≤2	–	≥4	
AMINOGLYCOSIDES									
A	Gentamicin	10 µg	≥15	13–14	≤12	≤4	8	≥16	
A	Tobramycin	10 µg	≥15	13–14	≤12	≤4	8	≥16	
B	Amikacin	30 µg	≥17	15–16	≤14	≤16	32	≥64	
O	Netilmicin	–	–	–	–	≤8	16	≥32	
TETRACYCLINES **(8)** Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. However, some organisms that are intermediate or resistant to tetracycline may be susceptible to doxycycline, minocycline, or both.									
B	Doxycycline	30 µg	≥13	10–12	≤9	≤4	8	≥16	
B	Minocycline	30 µg	≥16	13–15	≤12	≤4	8	≥16	
U	Tetracycline	30 µg	≥15	12–14	≤11	≤4	8	≥16	

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Table 2B-2. *Acinetobacter* spp. (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
FLUOROQUINOLONES									
A	Ciprofloxacin	5 µg	≥21	16–20	≤15	≤1	2	≥4	
A	Levofloxacin	5 µg	≥17	14–16	≤13	≤2	4	≥8	
O	Gatifloxacin	5 µg	≥18	15–17	≤14	≤2	4	≥8	
FOLATE PATHWAY ANTAGONISTS									
B	Trimethoprim-sulfamethoxazole	1.25/23.75 µg	≥ 16	11–15	≤10	≤2/38	–	≥4/76	

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; I, intermediate; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; R, resistant; S, susceptible.

Reference for Table 2B-2

1 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

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Table 2B-3. Zone Diameter and MIC Breakpoints for *Burkholderia cepacia* complex

Testing Conditions

Medium: Disk diffusion: MHA
Broth dilution: CAMHB
Agar dilution: MHA

Inoculum: **Broth culture** method or colony suspension, equivalent to a 0.5 McFarland standard

Incubation: 35°C±2°C; ambient air; 20–24 hours, all methods

Routine QC Recommendations (see Tables 4A-1 and 5A-1 for acceptable QC ranges)

Escherichia coli ATCC®* 25922 (for chloramphenicol, minocycline, and trimethoprim-sulfamethoxazole)
Pseudomonas aeruginosa ATCC® 27853

Refer to Tables 4A-2 and 5A-2 to select strains for routine QC of β-lactam combination agents.

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

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

General Comment

(1) For disk diffusion, test a maximum of 12 disks on a 150-mm plate and no more than 6 disks on a 100-mm plate; disks should be placed no less than 24 mm apart, center to center (see M02,[1] Subchapter 3.6). Each zone diameter should be clearly measurable; overlapping zones prevent accurate measurement. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Hold the Petri plate a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.

NOTE: Information in boldface type is new or modified since the previous edition.

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Table 2B-3. *Burkholderia cepacia* complex (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
β-LACTAM COMBINATION AGENTS									
O	Ticarcillin-clavulanate	–	–	–	–	≤16/2	32/2–64/2	≥128/2	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)									
B	Ceftazidime	30 µg	≥21	18–20	≤17	≤8	16	≥32	
CARBAPENEMS									
A	Meropenem	10 µg	≥20	16–19	≤15	≤4	8	≥16	
TETRACYCLINES									
B	Minocycline	30 µg	≥19	15–18	≤14	≤4	8	≥16	
FLUOROQUINOLONES									
A	Levofloxacin	–	–	–	–	≤2	4	≥8	
FOLATE PATHWAY ANTAGONISTS									
A	Trimethoprim-sulfamethoxazole	1.25/23.75 µg	≥16	11–15	≤10	≤2/38	–	≥4/76	
PHENICOLS									
C	Chloramphenicol	–	–	–	–	≤8	16	≥32	**(2)** Not routinely reported on isolates from the urinary tract.

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; I, intermediate; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; R, resistant; S, susceptible.

Reference for Table 2B-3

[1] CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

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Table 2B-4. Zone Diameter and MIC Breakpoints for *Stenotrophomonas maltophilia*

Testing Conditions

Medium: Disk diffusion: MHA
Broth dilution: CAMHB
Agar dilution: MHA

Inoculum: **Broth culture** method or colony suspension, equivalent to a 0.5 McFarland standard

Incubation: 35°C±2°C; ambient air; 20–24 hours, all methods

Routine QC Recommendations (see Tables 4A-1 and 5A-1 for acceptable QC ranges)

Escherichia coli ATCC®* 25922 (for chloramphenicol, minocycline, and trimethoprim-sulfamethoxazole)
Pseudomonas aeruginosa ATCC® 27853

Refer to Tables 4A-2 and 5A-2 to select strains for routine QC of β-lactam combination agents.

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

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

General Comment

(1) For disk diffusion, test a maximum of 12 disks on a 150-mm plate and no more than 6 disks on a 100-mm plate; disks should be placed no less than 24 mm apart, center to center (see M02,[1] Subchapter 3.6). Each zone diameter should be clearly measurable; overlapping zones prevent accurate measurement. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Hold the Petri plate a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.

NOTE: Information in boldface type is new or modified since the previous edition.

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
β-LACTAM COMBINATION AGENTS									
O	Ticarcillin-clavulanate	–	–	–	–	≤16/2	32/2–64/2	≥128/2	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)									
B	Ceftazidime	–	–	–	–	≤8	16	≥32	
TETRACYCLINES									
B	Minocycline	30 µg	≥19	15–18	≤14	≤4	8	≥16	
FLUOROQUINOLONES									
B	Levofloxacin	5 µg	≥17	14–16	≤13	≤2	4	≥8	

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Table 2B-4. *Stenotrophomonas maltophilia* (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
FOLATE PATHWAY ANTAGONISTS									
A	Trimethoprim-sulfamethoxazole	1.25/23.75 µg	≥ 16	11–15	≤ 10	≤2/38	–	≥4/76	
PHENICOLS									
C	Chloramphenicol	–	–	–	–	≤8	16	≥32	**(2)** Not routinely reported on isolates from the urinary tract.

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; I, intermediate; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; R, resistant; S, susceptible.

Reference for Table 2B-4

[1] CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

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Table 2B-5. MIC Breakpoints for Other Non-*Enterobacteriaceae* (Refer to General Comment 1)

Testing Conditions	
Medium:	Broth dilution: CAMHB Agar dilution: MHA
Inoculum:	**Broth culture** method or colony suspension, equivalent to a 0.5 McFarland standard
Incubation:	35°C ± 2°C; ambient air; 16–20 hours

Routine QC Recommendations (see Tables 4A-1 and 5A-1 for acceptable QC ranges)

Escherichia coli ATCC®* 25922 (for chloramphenicol, tetracyclines, sulfonamides, and trimethoprim-sulfamethoxazole)
Pseudomonas aeruginosa ATCC® 27853

Refer to Tables 4A-2 and 5A-2 to select strains for routine QC of β-lactam combination agents.

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

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

General Comments

(1) Other non-*Enterobacteriaceae* include *Pseudomonas* spp. (not *P. aeruginosa*) and other nonfastidious, glucose-nonfermenting, gram-negative bacilli, but exclude *P. aeruginosa, Acinetobacter* spp., *B. cepacia, B. mallei, B. pseudomallei,* and *S. maltophilia.* Refer to Tables 2B-2, 2B-3, and 2B-4 for testing of *Acinetobacter* spp., *B. cepacia* complex, and *S. maltophilia,* respectively, and CLSI document M45[1] for testing of *B. mallei, B. pseudomallei, Aeromonas* spp., and *Vibrio* spp.

(2) For other non-*Enterobacteriaceae,* the disk diffusion method has not been systematically studied. Therefore, for this organism group, disk diffusion testing is not recommended.

NOTE: Information in boldface type is new or modified since the previous edition.

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Table 2B-5. Other Non-*Enterobacteriaceae* (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
PENICILLINS									
O	Piperacillin	–	–	–	–	≤16	32–64	≥128	
β-LACTAM COMBINATION AGENTS									
B	Piperacillin-tazobactam	–	–	–	–	≤16/4	32/4–64/4	≥128/4	
O	Ticarcillin-clavulanate	–	–	–	–	≤16/2	32/2–64/2	≥128/2	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)									
A	Ceftazidime	–	–	–	–	≤8	16	≥32	
B	Cefepime	–	–	–	–	≤8	16	≥32	
C	Cefotaxime	–	–	–	–	≤8	16–32	≥64	
C	Ceftriaxone	–	–	–	–	≤8	16–32	≥64	
O	Cefoperazone	–	–	–	–	≤16	32	≥64	
O	Ceftizoxime	–	–	–	–	≤8	16–32	≥64	
O	Moxalactam	–	–	–	–	≤8	16–32	≥64	
MONOBACTAMS									
B	Aztreonam	–	–	–	–	≤8	16	≥32	
CARBAPENEMS									
B	Imipenem	–	–	–	–	≤4	8	≥16	
B	Meropenem	–	–	–	–	≤4	8	≥16	
AMINOGLYCOSIDES									
A	Gentamicin	–	–	–	–	≤4	8	≥16	
A	Tobramycin	–	–	–	–	≤4	8	≥16	
B	Amikacin	–	–	–	–	≤16	32	≥64	
O	Netilmicin	–	–	–	–	≤8	16	≥32	
TETRACYCLINES **(3)** Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. However, some organisms that are intermediate or resistant to tetracycline may be susceptible to doxycycline, minocycline, or both.									
U	Tetracycline	–	–	–	–	≤4	8	≥16	
O	Doxycycline	–	–	–	–	≤4	8	≥16	
O	Minocycline	–	–	–	–	≤4	8	≥16	
FLUOROQUINOLONES									
B	Ciprofloxacin	–	–	–	–	≤1	2	≥4	
B	Levofloxacin	–	–			≤2	4	≥8	
O	Gatifloxacin	–	–	–	–	≤2	4	≥8	
O	Lomefloxacin	–	–	–	–	≤2	4	≥8	
O	Norfloxacin	–	–	–	–	≤4	8	≥16	**(4)** For testing and reporting of urinary tract isolates only.
O	Ofloxacin	–	–	–	–	≤2	4	≥8	

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Table 2B-5. Other Non-*Enterobacteriaceae* (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
FOLATE PATHWAY ANTAGONISTS									
B	Trimethoprim-sulfamethoxazole	–	–	–	–	≤2/38	–	≥4/76	
U	Sulfonamides	–	–	–	–	≤256	–	≥512	**(5)** Sulfisoxazole can be used to represent any of the currently available sulfonamide preparations.
PHENICOLS									
C	Chloramphenicol	–	–	–	–	≤8	16	≥32	**(6)** Not routinely reported on isolates from the urinary tract.

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; I, intermediate; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; R, resistant; S, susceptible.

Reference for Table 2B-5

1 CLSI. *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria.* 3rd ed. CLSI guideline M45. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.

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Table 2C. Zone Diameter and MIC Breakpoints for *Staphylococcus* spp.

Testing Conditions

Medium: Disk diffusion: MHA
Broth dilution: CAMHB; CAMHB + 2% NaCl for oxacillin; CAMHB supplemented to 50 µg/mL calcium for daptomycin
Agar dilution: MHA; MHA + 2% NaCl for oxacillin. Agar dilution has not been validated for daptomycin.

Inoculum: Colony suspension, equivalent to a 0.5 McFarland standard

Incubation: 35°C ± 2°C; ambient air
Disk diffusion: 16–18 hours; 24 hours (CoNS and cefoxitin)
Dilution methods: 16–20 hours;
24 hours for oxacillin and vancomycin
Testing at temperatures above 35°C may not detect MRS.

Routine QC Recommendations (see Tables 4A**-1 and 5**A**-1** for acceptable QC ranges)

Disk diffusion:
S. aureus ATCC®* 25923

Dilution methods:
S. aureus ATCC® 29213

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

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

General Comments

(1) For disk diffusion, test a maximum of 12 disks on a 150-mm plate and no more than 6 disks on a 100-mm plate; disks should be placed no less than 24 mm apart, center to center (see M02,[1] Subchapter 3.6). Each zone diameter should be clearly measurable; overlapping zones prevent accurate measurement. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Hold the Petri plate a few inches above a black background illuminated with reflected light, except for linezolid, which should be read with transmitted light (plate held up to light source). The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter. For linezolid, any discernible growth within the zone of inhibition is indicative of resistance to the respective agent.

(2) For staphylococci when testing chloramphenicol, clindamycin, erythromycin, linezolid, tedizolid, and tetracycline by broth microdilution MIC, trailing growth can make end-point determination difficult. In such cases, read the MIC at the lowest concentration where the trailing begins. Tiny buttons of growth should be ignored (see M07,[2] Figures 3 and 4). With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, read the end point at the concentration in which there is ≥ 80% reduction in growth as compared to the control (see M07,[2] Figure 5).

(3) Historically, resistance to the penicillinase-stable penicillins (see Glossary I) has been referred to as "methicillin resistance" or "oxacillin resistance." MRSAs are those strains of *S. aureus* that express *mecA* or another mechanism of methicillin resistance, such as changes in affinity of penicillin-binding proteins for oxacillin (modified *S. aureus* strains).

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Table 2C. *Staphylococcus* spp. (Continued)

(4) Most oxacillin resistance is mediated by *mecA,* encoding the PBP2a (also called PBP2'). Isolates that test positive for *mecA* or PBP2a should be reported as oxacillin resistant.

Detection of oxacillin resistance in staphylococci is achieved by using specific methods as listed in Table 2C and further described in Table 3E.

Staphylococcus spp.	Acceptable Methods
S. aureus* and *S. lugdunensis	• **Cefoxitin MIC** • **Cefoxitin disk diffusion** • **Oxacillin MIC** • **Oxacillin salt agar (*S. aureus* only)**
S. pseudintermedius* and *S. schleiferi	• **Oxacillin MIC** • **Oxacillin disk diffusion**
CoNS* (except *S. lugdunensis, S. pseudintermedius, and S. schleiferi*).	• **Cefoxitin disk diffusion** • **Oxacillin MIC**

* **For non–*S. epidermidis* strains with oxacillin MICs between 0.5–2 µg/mL, see comment (16) for recommendations on testing for *mecA* or for PBP2a.**

Mechanisms of oxacillin resistance other than *mecA* are rare and include a novel *mecA* homologue, *mecC.*[3] MICs for strains with *mecC* are typically in the resistant range for cefoxitin and/or oxacillin; *mecC* resistance cannot be detected by tests directed at *mecA* or PBP2a.

(5) Oxacillin-resistant *S. aureus* and CoNS (MRS), are considered resistant to other β-lactam agents, ie, penicillins, β-lactam **combination agents**, cephems (with the exception of the cephalosporins with anti-MRSA activity), and carbapenems. This is because most cases of documented MRS infections have responded poorly to β-lactam therapy or because convincing clinical data that document clinical efficacy for those agents have not been presented.

(6) Routine testing of urine isolates of *Staphylococcus saprophyticus* is not advised, because infections respond to concentrations achieved in urine of antimicrobial agents commonly used to treat acute, uncomplicated UTIs (eg, nitrofurantoin, trimethoprim ± sulfamethoxazole, or a fluoroquinolone).

(7) For tests for β-lactamase production, oxacillin resistance and *mecA*-mediated oxacillin resistance using cefoxitin, reduced susceptibility to vancomycin, inducible clindamycin resistance, and high-level mupirocin resistance (*S. aureus* only), refer to Tables **3D,** 3E, 3F, 3G, and 3H, respectively.

NOTE: Information in boldface type is new or modified since the previous edition.

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Table 2C. *Staphylococcus* spp. (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
PENICILLINASE-LABILE PENICILLINS **(8)** Penicillin-susceptible staphylococci are susceptible to other β-lactam agents with established clinical efficacy for staphylococcal infections (including both penicillinase-labile and penicillinase-stable agents; see Glossary I). Penicillin-resistant staphylococci are resistant to penicillinase-labile penicillins.									
A	Penicillin	10 units	≥29	–	≤28	≤0.12	–	≥0.25	**(9)** Penicillin should be used to test the susceptibility of all staphylococci to all penicillinase-labile penicillins (see Glossary I). Penicillin-resistant strains of staphylococci produce β-lactamase. Perform a test(s) to detect β-lactamase production on staphylococci for which the penicillin MICs are ≤0.12 µg/mL or zone diameters ≥29 mm before reporting the isolate as penicillin susceptible. Rare isolates of staphylococci that contain genes for β-lactamase production may appear negative by β-lactamase tests. Consequently, for serious infections requiring penicillin therapy, laboratories should perform MIC tests and β-lactamase testing on all subsequent isolates from the same patient. PCR testing of the isolate for the *bla*Z β-lactamase gene may be considered. See Tables 3D and 3E. **(10)** For oxacillin-resistant staphylococci, report penicillin as resistant or do not report.
PENICILLINASE-STABLE PENICILLINS **(11)** Oxacillin (or cefoxitin) results can be applied to the other penicillinase-stable penicillins (cloxacillin, dicloxacillin, methicillin, and nafcillin). For agents with established clinical efficacy and considering site of infection and appropriate dosing, oxacillin (cefoxitin)-susceptible staphylococci can be considered susceptible to: • β-lactam **combination agents** (amoxicillin-clavulanate, ampicillin-sulbactam, piperacillin-tazobactam) • Oral cephems (cefaclor, cefdinir, cephalexin, cefpodoxime, cefprozil, cefuroxime, loracarbef) • Parenteral cephems including cephalosporins I, II, III, and IV (cefamandole, cefazolin, cefepime, cefmetazole, cefonicid, cefoperazone, cefotaxime, cefotetan, ceftizoxime, ceftriaxone, cefuroxime, ceftaroline, moxalactam) • Carbapenems (doripenem, ertapenem, imipenem, meropenem) Oxacillin-resistant staphylococci are resistant to all currently available β-lactam antimicrobial agents, with the exception of the newer cephalosporins with anti-MRSA activity. Thus, susceptibility or resistance to a wide array of β-lactam antimicrobial agents may be deduced from testing only penicillin and either cefoxitin or oxacillin. Testing of other β-lactam agents, except those with anti-MRSA activity, is not advised. See general comments (4) and (5). Additional explanation on the use of cefoxitin for prediction of *mecA*-mediated oxacillin resistance can be found in Subchapter 3.12 of M07[2] and Subchapter 3.9 of M02.[1]								

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Table 2C. *Staphylococcus* spp. (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
PENICILLINASE-STABLE PENICILLINS (Continued)									
A	Oxacillin (For *S. aureus* and *S. lugdunensis*)		–	–	–	≤2 (oxacillin)	–	≥4 (oxacillin)	For use with *S. aureus* and S. *lugdunensis*. **(12)** Oxacillin disk testing is not reliable. See cefoxitin for reporting oxacillin when testing cefoxitin as a surrogate agent.
		30 µg cefoxitin (surrogate test for oxacillin)	≥ 22	–	≤21	≤4 (cefoxitin)	–	≥8 (cefoxitin)	**(13)** Cefoxitin is tested as a surrogate for oxacillin. **Isolates that test resistant by cefoxitin MIC, cefoxitin disk, or oxacillin MIC should be reported as oxacillin resistant. If testing only cefoxitin,** report oxacillin susceptible or resistant based on the cefoxitin result. (**14)** Cefoxitin MIC and disk diffusion tests performed on media other than CAMHB or unsupplemented MHA do not reliably detect *mecA*-mediated resistance in isolates of S. *aureus* that do not grow **well** on these media (eg, small colony variants). Testing for PBP2a using induced growth (ie, growth taken from the zone margin surrounding a cefoxitin disk on either BMHA or a blood agar plate after 24 hours incubation in 5% CO_2) or *mecA* should be done. Isolates that test either *mecA* negative or PBP2a negative or cefoxitin susceptible should be reported as oxacillin susceptible. See general comments (4) and (5) and comments (8) and (11).

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Table 2C. *Staphylococcus* spp. (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
PENICILLINASE-STABLE PENICILLINS (Continued)									
A	Oxacillin (For *S. pseudintermedius* **and *S. schleiferi***)	1 µg oxacillin	≥18	–	≤17	≤0.25	–	≥0.5	**(15)** Neither cefoxitin MIC nor cefoxitin disk tests are reliable for detecting *mecA*-mediated resistance in *S. pseudintermedius* **and *S. schleiferi.***
A	Oxacillin (For CoNS except *S. lugdunensis, S. pseudintermedius, and* ***S. schleiferi***)	–	–	–	–	≤0.25 (oxacillin)	–	≥0.5 (oxacillin)	**(16) *S. epidermidis* isolates with oxacillin MIC ≥0.5 µg/mL should be reported as oxacillin resistant. However, oxacillin MIC breakpoints may overcall resistance for some CoNS, because some non-*S. epidermidis* strains for which the oxacillin MICs are 0.5–2 µg/mL lack *mecA*. Non-*S. epidermidis* isolates from serious infections with MICs in this range may be tested for *mecA* or for PBP2a. Isolates that test *mecA* or PBP2a negative should be reported as oxacillin susceptible.**
		30 µg cefoxitin (surrogate test for oxacillin)	≥25	–	≤24	–	–	–	See general comment (5) and comments (8), (11), and (13).
CEPHEMS (PARENTERAL)									
B	Ceftaroline	30 µg	≥24	21–23	≤20	≤1	2	≥4	**(17)** For reporting against *S. aureus* only, including MRSA. **(18)** Breakpoints are based on a dosage regimen of 600 mg every 12 h.

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Table 2C. *Staphylococcus* spp. (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
GLYCOPEPTIDES									
(19) For *S. aureus,* vancomycin-susceptible isolates may become vancomycin intermediate during the course of prolonged therapy.									
B	Vancomycin (For *S. aureus*)	–	–	–	–	≤2	4–8	≥16	**(20)** MIC tests should be performed to determine the susceptibility of all isolates of staphylococci to vancomycin. The disk test does not differentiate vancomycin-susceptible isolates of *S. aureus* from vancomycin-intermediate isolates, nor does the test differentiate among vancomycin-susceptible, -intermediate, and -resistant isolates of CoNS, all of which give similar size zones of inhibition. **(21)** Send any *S. aureus* for which the vancomycin is ≥8 µg/mL to a referral laboratory. See Appendix A. Also refer to Table 3**F** for *S. aureus,* Subchapter 3.12 in M07,[2] and Subchapter 3.9 in M02.[1]
B	Vancomycin (For CoNS)	–	–	–	–	≤4	8–16	≥32	See comment (20). **(22)** Send any CoNS for which the vancomycin MIC is ≥32 µg/mL to a referral laboratory. See Appendix A. See also Subchapter 3.12 in M07[2] and Subchapter 3.9 in M02.[1]
Inv.	Teicoplanin	–	–	–	–	≤8	16	≥32	
LIPOGLYCOPEPTIDES									
C	**Dalbavancin**	**–**	**–**	**–**	**–**	**≤0.25**	**–**	**–**	**See comment (17).**
C	Oritavancin	–	–	–	–	≤0.12	–	–	See comment (17).
C	Telavancin	–	–	–	–	≤0.12	–	–	See comment (17).
LIPOPEPTIDES									
B	Daptomycin	–	–	–	–	≤1	–	–	**(23)** Daptomycin should not be reported for isolates from the respiratory tract.

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Table 2C. *Staphylococcus* spp. (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
AMINOGLYCOSIDES									
(24) For staphylococci that test susceptible, **gentamicin is** used only in combination with other active agents that test susceptible.									
C	Gentamicin	10 µg	≥15	13–14	≤12	≤4	8	≥16	
MACROLIDES									
(25) Not routinely reported on organisms isolated from the urinary tract.									
A	Azithromycin or	15 µg	≥18	14–17	≤13	≤2	4	≥8	
A	clarithromycin or	15 µg	≥18	14–17	≤13	≤2	4	≥8	
A	erythromycin	15 µg	≥23	14–22	≤13	≤0.5	1–4	≥8	
O	Telithromycin	15 µg	≥22	19–21	≤18	≤1	2	≥4	
O	Dirithromycin	15 µg	≥19	16–18	≤15	≤2	4	≥8	
TETRACYCLINES									
(26) Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. However, some organisms that are intermediate or resistant to tetracycline may be susceptible to doxycycline, minocycline, or both.									
B	Tetracycline	30 µg	≥19	15–18	≤14	≤4	8	≥16	
B	Doxycycline	30 µg	≥16	13–15	≤12	≤4	8	≥16	
B	Minocycline	30 µg	≥19	15–18	≤14	≤4	8	≥16	See comment (25).
FLUOROQUINOLONES									
(27) *Staphylococcus* spp. may develop resistance during prolonged therapy with quinolones. Therefore, isolates that are initially susceptible may become resistant within 3 to 4 days after initiation of therapy. Testing of repeat isolates may be warranted.									
C	Ciprofloxacin or	5 µg	≥21	16–20	≤15	≤1	2	≥4	
C	levofloxacin	5 µg	≥19	16–18	≤15	≤1	2	≥4	
C	Moxifloxacin	5 µg	≥24	21–23	≤20	≤0.5	1	≥2	
O	Enoxacin	10 µg	≥18	15–17	≤14	≤2	4	≥8	**(28)** For testing and reporting of urinary tract isolates only.
O	Gatifloxacin	5 µg	≥23	20–22	≤19	≤0.5	1	≥2	
O	Grepafloxacin	5 µg	≥18	15–17	≤14	≤1	2	≥4	
O	Lomefloxacin	10 µg	≥22	19–21	≤18	≤2	4	≥8	
O	Norfloxacin	10 µg	≥17	13–16	≤12	≤4	8	≥16	See comment (28).
O	Ofloxacin	5 µg	≥18	15–17	≤14	≤1	2	≥4	
O	Sparfloxacin	5 µg	≥19	16–18	≤15	≤0.5	1	≥2	
Inv.	Fleroxacin	5 µg	≥19	16–18	≤15	≤2	4	≥8	
NITROFURANTOINS									
U	Nitrofurantoin	300 µg	≥17	15–16	≤14	≤32	64	≥128	

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Table 2C. *Staphylococcus* spp. (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
LINCOSAMIDES									
A	Clindamycin	2 µg	≥21	15–20	≤14	≤0.5	1–2	≥4	**(29)** Inducible clindamycin resistance can be detected by disk diffusion using the D-zone test or by broth microdilution (see Table 3**G**, Subchapter 3.9 in M02,[1] and Subchapter 3.12 in M07[2]). See comment (25).
FOLATE PATHWAY ANTAGONISTS									
A	Trimethoprim-sulfamethoxazole	1.25/23.75 µg	≥16	11–15	≤10	≤2/38	–	≥4/76	
U	Sulfonamides	250 or 300 µg	≥17	13–16	≤12	≤256	–	≥512	**(30)** Sulfisoxazole can be used to represent any of the currently available sulfonamide preparations.
U	Trimethoprim	5 µg	≥16	11–15	≤10	≤8	–	≥16	
PHENICOLS									
C	Chloramphenicol	30 µg	≥18	13–17	≤12	≤8	16	≥32	See comment (25).
ANSAMYCINS									
B	Rifampin	5 µg	≥20	17–19	≤16	≤1	2	≥4	**(31)** ***Rx:*** Rifampin should not be used alone for antimicrobial therapy.
STREPTOGRAMINS									
O	Quinupristin-dalfopristin	15 µg	≥19	16–18	≤15	≤1	2	≥4	**(32)** For reporting against methicillin-susceptible *S. aureus.*
OXAZOLIDINONES									
B	Linezolid	30 µg	≥21	–	≤20	≤4	–	≥8	**(33)** When testing linezolid, disk diffusion zones should be examined using transmitted light. Organisms with resistant results by disk diffusion should be confirmed using an MIC method.
B	Tedizolid	–	–	–	–	≤0.5	1	≥2	See comment (17).

Abbreviations: ATCC®, American Type Culture Collection; BMHA, blood Mueller-Hinton agar; CAMHB, cation-adjusted Mueller-Hinton broth; CoNS, coagulase-negative staphylococci; I, intermediate; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; MRS, methicillin-resistant staphylococci; MRSA, methicillin-resistant *S. aureus;* PBP, penicillin-binding protein; PCR, polymerase chain reaction; QC, quality control; R, resistant; S, susceptible; UTI, urinary tract infection.

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Table 2C. *Staphylococcus* spp. (Continued)

References for Table 2C

1 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

2 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

3 García-Álvarez L, Holden MT, Lindsay H, et al. Methicillin-resistant *Staphylococcus aureus* with a novel *mecA* homologue in human and bovine populations in the UK and Denmark: a descriptive study. *Lancet Infect Dis*. 2011;11(8):595-603.

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Table 2D. Zone Diameter and MIC Breakpoints for *Enterococcus* spp.

Testing Conditions	
Medium:	Disk diffusion: MHA Broth dilution: CAMHB; CAMHB supplemented to 50 µg/mL calcium for daptomycin Agar dilution: MHA; agar dilution has not been validated for daptomycin
Inoculum:	**Broth culture** method or colony suspension, equivalent to a 0.5 McFarland standard
Incubation:	35°C ± 2°C; ambient air Disk diffusion: 16–18 hours Dilution methods: 16–20 hours All methods: 24 hours for vancomycin

Routine QC Recommendations (see Tables 4A-1 and 5A-1 for acceptable QC ranges)

Disk diffusion:
S. aureus ATCC®* 25923

Dilution methods:
E. faecalis ATCC® 29212

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

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

Refer to Tables **3F** and **3I** for additional testing recommendations, reporting suggestions, and QC.

General Comments

(1) For disk diffusion, test a maximum of 12 disks on a 150-mm plate and no more than 6 disks on a 100-mm plate; disks should be placed no less than 24 mm apart, center to center (see M02,[1] Subchapter 3.6). Each zone diameter should be clearly measurable; overlapping zones prevent accurate measurement. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Hold the Petri plate a few inches above a black background illuminated with reflected light, except for vancomycin, which should be read with transmitted light (plate held up to light source). The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. Any discernible growth within the zone of inhibition indicates vancomycin resistance.

(2) For enterococci when testing chloramphenicol, erythromycin, linezolid, tedizolid, and tetracycline by broth microdilution MIC, trailing growth can make end-point determination difficult. In such cases, read the MIC at the lowest concentration where the trailing begins. Tiny buttons of growth should be ignored (see M07,[2] Figures 3 and 4).

(3) **WARNING:** For *Enterococcus* spp., aminoglycosides (except for high-level resistance testing), cephalosporins, clindamycin, and trimethoprim-sulfamethoxazole may appear active *in vitro,* but they are not effective clinically, and isolates should not be reported as susceptible.

(4) Synergy between ampicillin, penicillin, or vancomycin and an aminoglycoside can be predicted for enterococci by using a high-level aminoglycoside (gentamicin and streptomycin) test (see Table 3**I**).

NOTE: Information in boldface type is new or modified since the previous edition.

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Table 2D. *Enterococcus* spp. (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
PENICILLINS									
A A	Penicillin Ampicillin	10 units 10 µg	≥15 ≥17	– –	≤14 ≤16	≤8 ≤8	– –	≥16 ≥16	**(5)** The results of ampicillin susceptibility tests should be used to predict the activity of amoxicillin. Ampicillin results may be used to predict susceptibility to amoxicillin-clavulanate, ampicillin-sulbactam, and piperacillin-tazobactam among non–β-lactamase-producing enterococci. Ampicillin susceptibility can be used to predict imipenem susceptibility, providing the species is confirmed to be *E. faecalis*. **(6)** Enterococci susceptible to penicillin are predictably susceptible to ampicillin, amoxicillin, ampicillin-sulbactam, amoxicillin-clavulanate, and piperacillin-tazobactam for non–β-lactamase-producing enterococci. However, enterococci susceptible to ampicillin cannot be assumed to be susceptible to penicillin. If penicillin results are needed, testing of penicillin is required. **(7) *Rx:*** Combination therapy with ampicillin, penicillin, or vancomycin (for susceptible strains only), plus an aminoglycoside, is usually indicated for serious enterococcal infections, such as endocarditis, unless high-level resistance to both gentamicin and streptomycin is documented; such combinations are predicted to result in synergistic killing of the *Enterococcus*. **For strains with low-level penicillin or ampicillin resistance when combination therapy with a β-lactam is being considered, also see additional testing and reporting information in Table 3I.[3]** **(8)** Penicillin or ampicillin resistance among enterococci due to β-lactamase production has been reported very rarely. Penicillin or ampicillin resistance due to β-lactamase production is not reliably detected with routine disk or dilution methods, but is detected using a direct, nitrocefin-based β-lactamase test. Because of the rarity of β-lactamase-positive enterococci, this test does not need to be performed routinely but can be used in selected cases. A positive β-lactamase test predicts resistance to penicillin as well as amino- and ureidopenicillins (see Glossary I).

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Table 2D. *Enterococcus* spp. (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
GLYCOPEPTIDES									
B	Vancomycin	30 µg	≥17	15–16	≤14	≤4	8–16	≥32	**(9)** When testing vancomycin against enterococci, plates should be held a full 24 hours for accurate detection of resistance. Zones should be examined using transmitted light; the presence of a haze or any growth within the zone of inhibition indicates resistance. Organisms with intermediate zones should be tested by an MIC method as described in M07.[2] For isolates for which the vancomycin MICs are 8–16 µg/mL, perform biochemical tests for identification as listed under the "Vancomycin MIC ≥ 8 µg/mL" test found in Table 3**F**. See general comment (4) and comment (7).
Inv.	Teicoplanin	30 µg	≥14	11–13	≤10	≤8	16	≥32	
LIPOGLYCOPEPTIDES									
C	**Dalbavancin**	**–**	**–**	**–**	**–**	**≤0.25**	**–**	**–**	**(10) For reporting against vancomycin-susceptible *E. faecalis*.**
C	Oritavancin	–	–	–	–	≤0.12	–	–	See comment (10).
C	Telavancin	–	–	–	–	≤0.25	–	–	See comment (10).
LIPOPEPTIDES									
B	Daptomycin	–	–	–	–	≤4	–	–	**(11)** Daptomycin should not be reported for isolates from the respiratory tract.
MACROLIDES									
O	Erythromycin	15 µg	≥23	14–22	≤13	≤0.5	1–4	≥8	**(12)** Not routinely reported on isolates from the urinary tract.
TETRACYCLINES **(13)** Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. However, some organisms that are intermediate or resistant to tetracycline may be susceptible to doxycycline, minocycline, or both.									
U	Tetracycline	30 µg	≥19	15–18	≤14	≤4	8	≥16	
O	Doxycycline	30 µg	≥16	13–15	≤12	≤4	8	≥16	
O	Minocycline	30 µg	≥19	15–18	≤14	≤4	8	≥16	
FLUOROQUINOLONES									
U	Ciprofloxacin	5 µg	≥21	16–20	≤15	≤1	2	≥4	
U	Levofloxacin	5 µg	≥17	14–16	≤13	≤2	4	≥8	
O	Gatifloxacin	5 µg	≥18	15–17	≤14	≤2	4	≥8	
O	Norfloxacin	10 µg	≥17	13–16	≤12	≤4	8	≥16	**(14)** For testing and reporting of urinary tract isolates only.
NITROFURANTOINS									
U	Nitrofurantoin	300 µg	≥17	15–16	≤14	≤32	64	≥128	

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Table 2D. *Enterococcus* spp. (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
ANSAMYCINS									
O	Rifampin	5 µg	≥20	17–19	≤16	≤1	2	≥4	**(15)** ***Rx:*** Rifampin should not be used alone for antimicrobial therapy.
FOSFOYCINS									
U	Fosfomycin	200 µg	≥16	13–15	≤12	≤64	128	≥256	**(16)** For testing and reporting of *E. faecalis* urinary tract isolates only. **(17)** The approved MIC testing method is agar dilution. Agar media should be supplemented with 25 µg/mL of glucose-6-phosphate. Broth dilution testing should not be performed. **(18)** The 200-µg fosfomycin disk contains 50 µg of glucose-6-phosphate.
PHENICOLS									
O	Chloramphenicol	30 µg	≥18	13–17	≤12	≤8	16	≥32	See comment (12).
STREPTOGRAMINS									
O	Quinupristin-dalfopristin	15 µg	≥19	16–18	≤15	≤1	2	≥4	**(19)** For reporting against vancomycin-resistant *Enterococcus faecium*.
OXAZOLIDINONES									
B	Linezolid	30 µg	≥23	21–22	≤20	≤2	4	≥8	
B	Tedizolid	–	–	–	–	≤0.5	–	–	**(20)** For reporting against *E. faecalis* only.

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; I, intermediate; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; R, resistant; S, susceptible.

References for Table 2D

1 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

2 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

3 Murray BE, Arias CA, Nannini EC. Glycopeptides (vancomycin and teicoplanin), streptogramins (quinupristin-dalfopristin), lipopeptides (daptomycin), and lipoglycopeptides (telavancin). In: Bennett JE, Dolin R, Blaser MJ. *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*. 8th ed. Philadelphia, PA: Elsevier Saunders, 2015:377-400.

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Table 2E. Zone Diameter and MIC Breakpoints for *Haemophilus influenzae* and *Haemophilus parainfluenzae*

Testing Conditions

Medium: Disk diffusion: HTM
Broth dilution: HTM broth

Inoculum: Colony suspension, equivalent to a 0.5 McFarland standard prepared using colonies from an overnight (preferably 20- to 24-hour) chocolate agar plate (see comment [2])

Incubation: 35°C ± 2°C
Disk diffusion: 5% CO_2; 16–18 hours
Broth dilution: ambient air; 20–24 hours

Routine QC Recommendations (see Tables 4A-**1**, 4B, 5A-**1,** and 5B for acceptable QC ranges)

H. influenzae ATCC®* 49247
H. influenzae ATCC® 49766

Use either *H. influenzae* ATCC® 49247 or *H. influenzae* ATCC® 49766 or both of these strains, based on the antimicrobial agents to be tested. Neither strain has QC ranges for all agents that might be tested against *H. influenzae* or *H. parainfluenzae*.

E. coli ATCC® 35218 (when testing amoxicillin-clavulanate)

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

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

General Comments

(1) *Haemophilus* spp., as used in this table, includes only *H. influenzae* and *H. parainfluenzae.* See CLSI document M45[1] for testing and reporting recommendations for other species of *Haemophilus*.

(2) The 0.5 McFarland suspension contains approximately 1 to 4 × 10^8 CFU/mL. Use care in preparing this suspension, because higher inoculum concentrations may lead to false-resistant results with some β-lactam antimicrobial agents, particularly when β-lactamase–producing strains of *H. influenzae* are tested.

(3) For disk diffusion, test a maximum of 9 disks on a 150-mm plate and 4 disks on a 100-mm plate. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Hold the Petri plate a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.

(4) For isolates of *H. influenzae* from CSF, only results of testing with ampicillin, any of the 3rd-generation cephalosporins listed below, chloramphenicol, and meropenem are appropriate to report.

(5) Amoxicillin-clavulanate, azithromycin, cefaclor, cefdinir, cefixime, cefpodoxime, cefprozil, cefuroxime, and clarithromycin are used as empiric therapy for respiratory tract infections due to *Haemophilus* spp. The results of susceptibility tests with these antimicrobial agents are often not necessary for management of individual patients.

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Table 2E. *Haemophilus influenzae* and *Haemophilus parainfluenzae* (Continued)

(6) To make HTM: Prepare a fresh hematin stock solution by dissolving 50 mg of hematin powder in 100 mL of 0.01 mol/L NaOH with heat and stirring until the powder is thoroughly dissolved. Add 30 mL of the hematin stock solution and 5 g of yeast extract to 1 L of MHA, and autoclave. After autoclaving and cooling, add 3 mL of an NAD stock solution (50 mg of NAD dissolved in 10 mL of distilled water, filter sterilized) aseptically.

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
PENICILLINS									
A	Ampicillin	10 µg	≥22	19–21	≤18	≤1	2	≥4	See general comment (4). **(7)** The results of ampicillin susceptibility tests should be used to predict the activity of amoxicillin. The majority of isolates of *H. influenzae* that are resistant to ampicillin and amoxicillin produce a TEM-type β-lactamase. In most cases, a direct β-lactamase test can provide a rapid means of detecting resistance to ampicillin and amoxicillin. **(8)** Rare BLNAR strains of *H. influenzae* should be considered resistant to amoxicillin-clavulanate, ampicillin-sulbactam, cefaclor, cefamandole, cefetamet, cefonicid, cefprozil, cefuroxime, loracarbef, and piperacillin-tazobactam, despite apparent *in vitro* susceptibility of some BLNAR strains to these agents.
β-LACTAM COMBINATION AGENTS									
B	Ampicillin-sulbactam	10/10 µg	≥20	–	≤19	≤2/1	–	≥4/2	See comment (8).
C	Amoxicillin-clavulanate	20/10 µg	≥20	–	≤19	≤4/2	–	≥8/4	See general comment (5) and comment (8).
O	Piperacillin-tazobactam	100/10 µg	≥21	–	–	≤1/4	–	≥2/4	See comment (8).
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)									
B B B	Cefotaxime or ceftazidime or ceftriaxone	30 µg 30 µg 30 µg	≥26 ≥26 ≥26	– – –	– – –	≤2 ≤2 ≤2	– – –	– – –	See general comment (4).
C	Cefuroxime	30 µg	≥20	17–19	≤16	≤4	8	≥16	See general comment (5) and comment (8).
C	Ceftaroline	30 µg	≥30	–	–	≤0.5	–	–	**(9)** For *H. influenzae* only. **(10)** Breakpoints are based on a dosage regimen of 600 mg every 12 h.
O	Cefonicid	30 µg	≥20	17–19	≤16	≤4	8	≥16	See comment (8).

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Table 2E. *Haemophilus influenzae* and *Haemophilus parainfluenzae* (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.) (Continued)									
O	Cefamandole	–	–	–	–	≤4	8	≥16	See comment (8).
O	Cefepime	30 µg	≥26	–	–	≤2	–	–	
O	Ceftizoxime	30 µg	≥26	–	–	≤2	–	–	See general comment (4).
CEPHEMS (ORAL)									
C	Cefaclor	30 µg	≥20	17–19	≤16	≤8	16	≥32	See general comment (5) and comment (8).
C	Cefprozil	30 µg	≥18	15–17	≤14	≤8	16	≥32	
C	Cefdinir or	5 µg	≥20	–	–	≤1	–	–	See general comment (5).
C	cefixime or	5 µg	≥21	–	–	≤1	–	–	
C	cefpodoxime	10 µg	≥21	–	–	≤2	–	–	
C	Cefuroxime	30 µg	≥20	17–19	≤16	≤4	8	≥16	See general comment (5) and comment (8).
O	Loracarbef	30 µg	≥19	16–18	≤15	≤8	16	≥32	See general comment (5) and comment (8).
O	Ceftibuten	30 µg	≥28	–	–	≤2	–	–	
Inv.	Cefetamet	10 µg	≥18	15–17	≤14	≤4	8	≥16	See comment (8).
MONOBACTAMS									
C	Aztreonam	30 µg	≥26	–	–	≤2	–	–	
CARBAPENEMS									
B	Meropenem	10 µg	≥20	–	–	≤0.5	–	–	See general comment (4).
C	Ertapenem or	10 µg	≥19	–	–	≤0.5	–	–	
C	imipenem	10 µg	≥16	–	–	≤4	–	–	
O	Doripenem	10 µg	≥16	–	–	≤1	–	–	
MACROLIDES									
C	Azithromycin	15 µg	≥12	–	–	≤4	–	–	See general comment (5).
C	Clarithromycin	15 µg	≥13	11–12	≤10	≤8	16	≥32	See general comment (5).
KETOLIDES									
O	Telithromycin	15 µg	≥15	12–14	≤11	≤4	8	≥16	See general comment (5).
TETRACYCLINES **(11)** Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline.									
C	Tetracycline	30 µg	≥29	26–28	≤25	≤2	4	≥8	
FLUOROQUINOLONES									
B	Ciprofloxacin or	5 µg	≥21	–	–	≤1	–	–	
B	levofloxacin or	5 µg	≥17	–	–	≤2	–	–	
B	moxifloxacin	5 µg	≥18	–	–	≤1	–	–	
O	Gemifloxacin	5 µg	≥18	–	–	≤0.12	–	–	
O	Gatifloxacin	5 µg	≥18	–	–	≤1	–	–	
O	Grepafloxacin	5 µg	≥24	–	–	≤0.5	–	–	
O	Lomefloxacin	10 µg	≥22	–	–	≤2	–	–	
O	Ofloxacin	5 µg	≥16	–	–	≤2	–	–	
O	Sparfloxacin	–	–	–	–	≤0.25	–	–	

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Table 2E. *Haemophilus influenzae* and *Haemophilus parainfluenzae* (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
FLUOROQUINOLONES (Continued)									
O	Trovafloxacin	10 µg	≥22	–	–	≤1	–	–	
Inv.	Fleroxacin	5 µg	≥19	–	–	≤2	–	–	
FOLATE PATHWAY ANTAGONISTS									
C	Trimethoprim-sulfamethoxazole	1.25/23.75 µg	≥16	11–15	≤10	≤0.5/9.5	1/19–2/38	≥4/76	
PHENICOLS									
C	Chloramphenicol	30 µg	≥29	26–28	≤25	≤2	4	≥8	See general comment (4). **(12)** Not routinely reported on isolates from the urinary tract.
ANSAMYCINS									
C	Rifampin	5 µg	≥20	17–19	≤16	≤1	2	≥4	**(13)** May be appropriate only for prophylaxis of case contacts. These breakpoints do not apply to therapy of patients with invasive *H. influenzae* disease.

Abbreviations: ATCC®, American Type Culture Collection; BLNAR, β-lactamase negative, ampicillin-resistant; CFU, colony-forming unit(s); CSF, cerebrospinal fluid; HTM, *Haemophilus* test medium; I, intermediate; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; NAD, nicotinamide adenine dinucleotide; QC, quality control; R, resistant; S, susceptible.

Reference for Table 2E

1 CLSI. *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria.* 3rd ed. CLSI guideline M45. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.

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Table 2F. Zone Diameter and MIC Breakpoints for *Neisseria gonorrhoeae*

Testing Conditions

Medium: Disk diffusion: GC agar base and 1% defined growth supplement. (The use of a cysteine-free growth supplement is not required for disk diffusion testing.)
Agar dilution: GC agar base and 1% defined growth supplement. (The use of a cysteine-free growth supplement is required for agar dilution tests with carbapenems and clavulanate. Cysteine-containing defined growth supplement does not significantly alter dilution test results with other drugs.)

Inoculum: Colony suspension, equivalent to a 0.5 McFarland standard prepared in MHB or 0.9% phosphate-buffered saline, pH 7, using colonies from an overnight (20- to 24-hour) chocolate agar plate incubated in 5% CO_2

Incubation: 36°C ± 1°C (do not exceed 37°C); 5% CO_2; all methods, 20–24 hours

Routine QC Recommendations (see Tables 4B and 5C for acceptable QC ranges)

N. gonorrhoeae ATCC®* 49226

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

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

General Comments

(1) For disk diffusion, test a maximum of 9 disks on a 150-mm plate and 4 disks on a 100-mm plate. For some agents, eg, fluoroquinolones or cephalosporins, only 2 to 3 disks may be tested per plate. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Hold the Petri plate a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth.

(2) The clinical effectiveness of cefmetazole, cefotetan, cefoxitin, and spectinomycin for treating infections due to organisms that produce intermediate results with these agents is unknown.

(3) For disk diffusion testing of *N. gonorrhoeae,* an intermediate result for an antimicrobial agent indicates either a technical problem that should be resolved by repeat testing or a lack of clinical experience in treating infections due to organisms with these zones. Strains with intermediate zones to agents other than cefmetazole, cefotetan, cefoxitin, and spectinomycin have a documented lower clinical cure rate (85% to 95%) compared with > 95% for susceptible strains.

(4) The recommended medium for testing *N. gonorrhoeae* consists of GC agar to which a 1% defined growth supplement (1.1 g L-cystine, 0.03 g guanine HCl, 0.003 g thiamine HCl, 0.013 g para-aminobenzoic acid, 0.01 g B12, 0.1 g cocarboxylase, 0.25 g NAD, 1 g adenine, 10 g L-glutamine, 100 g glucose, 0.02 g ferric nitrate, 25.9 g L-cysteine HCl [in 1 L H_2O]) is added after autoclaving.

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Table 2F. *Neisseria gonorrhoeae* (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
PENICILLINS									
O	Penicillin	10 units	≥47	27–46	≤26	≤0.06	0.12–1	≥2	See general comment (3). **(5)** A positive β-lactamase test predicts resistance to penicillin, ampicillin, and amoxicillin. **(6)** A β-lactamase test detects one form of penicillin resistance in *N. gonorrhoeae* and also may be used to provide epidemiological information. Strains with chromosomally mediated resistance can be detected only by the disk diffusion method or the agar dilution MIC method. **(7)** Gonococci that produce zones of inhibition of ≤19 mm around a 10-unit penicillin disk are likely to be β-lactamase–producing strains. However, the β-lactamase test remains preferable to other susceptibility methods for rapid, accurate recognition of this plasmid-mediated penicillin resistance.
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)									
A	Ceftriaxone	30 µg	≥35	–	–	≤0.25	–	–	
O	Cefoxitin	30 µg	≥28	24–27	≤23	≤2	4	≥8	See general comment (2).
O	Cefuroxime	30 µg	≥31	26–30	≤25	≤1	2	≥4	See general comment (3).
O	Cefepime	30 µg	≥31	–	–	≤0.5	–	–	
O	Cefmetazole	30 µg	≥33	28–32	≤27	≤2	4	≥8	See general comment (2).
O	Cefotaxime	30 µg	≥31	–	–	≤0.5	–	–	
O	Cefotetan	30 µg	≥26	20–25	≤19	≤2	4	≥8	See general comment (2).
O	Ceftazidime	30 µg	≥31	–	–	≤0.5	–	–	
O	Ceftizoxime	30 µg	≥38	–	–	≤0.5	–	–	
CEPHEMS (ORAL)									
A	Cefixime	5 µg	≥ 31	–	–	≤ 0.25	–	–	
O	Cefpodoxime	10 µg	≥ 29	–	–	≤0.5	–	–	
Inv.	Cefetamet	10 µg	≥ 29	–	–	≤0.5	–	–	

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Table 2F. *Neisseria gonorrhoeae* (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
TETRACYCLINES **(8)** Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline.									
A	Tetracycline	30 µg	≥38	31–37	≤30	≤0.25	0.5–1	≥2	**(9)** Gonococci with 30-µg tetracycline disk zone diameters of ≤19 mm usually indicate a plasmid-mediated tetracycline-resistant *N. gonorrhoeae* isolate. Resistance in these strains should be confirmed by a dilution test (MIC ≥16 µg/mL).
FLUOROQUINOLONES See general comment (3).									
A	Ciprofloxacin	5 µg	≥41	28–40	≤27	≤ 0.06	0.12–0.5	≥1	
O	Enoxacin	10 µg	≥36	32–35	≤31	≤ 0.5	1	≥2	
O	Lomefloxacin	10 µg	≥38	27–37	≤26	≤ 0.12	0.25–1	≥2	
O	Ofloxacin	5 µg	≥31	25–30	≤24	≤ 0.25	0.5–1	≥2	
Inv.	Fleroxacin	5 µg	≥35	29–34	≤28	≤ 0.25	0.5	≥1	
AMINOCYCLITOLS									
O	Spectinomycin	100 µg	≥18	15–17	≤14	≤ 32	64	≥128	See general comment (2).

Abbreviations: ATCC®, American Type Culture Collection; I, intermediate; MHB, Mueller-Hinton broth; MIC, minimal inhibitory concentration; QC, quality control; NAD, nicotinamide adenine dinucleotide; R, resistant; S, susceptible.

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Table 2G. Zone Diameter and MIC Breakpoints for *Streptococcus pneumoniae*

Testing Conditions

Medium: Disk diffusion: MHA with 5% sheep blood
Broth dilution: CAMHB with LHB (2.5% to 5% v/v)
(see M07[1] for instructions for preparation of LHB)
Agar dilution: MHA with sheep blood (5% v/v); recent studies using the agar dilution method have not been performed and reviewed by the subcommittee.

Inoculum: Colony suspension, equivalent to a 0.5 McFarland standard, prepared using colonies from an overnight (18- to 20-hour) sheep blood agar plate

Incubation: 35°C ± 2°C
Disk diffusion: 5% CO_2; 20–24 hours
Dilution methods: ambient air; 20–24 hours (CO_2 if necessary for growth with agar dilution)

Routine QC Recommendations (see Tables 4B and 5B for acceptable QC ranges)

S. pneumoniae ATCC®* 49619

Disk diffusion: deterioration of oxacillin disk content is best assessed with *S. aureus* ATCC® 25923, with an acceptable range of 18–24 mm on unsupplemented MHA.

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

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

General Comments

(1) For disk diffusion, test a maximum of 9 disks on a 150-mm plate and 4 disks on a 100-mm plate. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Do not measure the zone of inhibition of hemolysis. Measure the zones from the upper surface of the agar illuminated with reflected light, with the cover removed. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.

(2) For pneumococci when testing chloramphenicol, clindamycin, erythromycin, linezolid, tedizolid, and tetracycline by broth microdilution MIC, trailing growth can make end-point determination difficult. In such cases, read the MIC at the lowest concentration where the trailing begins. Tiny buttons of growth should be ignored (see M07,[1] Figures 3 and 4). With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, read the end point at the concentration in which there is ≥ 80% reduction in growth as compared to the control (see M07,[1] Figure 5).

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

(4) For *S. pneumoniae* isolated from CSF, penicillin and cefotaxime, ceftriaxone, or meropenem should be tested by a reliable MIC method (such as that described in M07[1]) and reported routinely. Such isolates can also be tested against vancomycin using the MIC or disk diffusion method.

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Table 2G. *Streptococcus pneumoniae* (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
PENICILLINS **(5)** For nonmeningitis isolates, a penicillin MIC of ≤0.06 µg/mL (or oxacillin zone ≥20 mm) can predict susceptibility to the following β-lactams: ampicillin (oral or parenteral), ampicillin-sulbactam, amoxicillin, amoxicillin-clavulanate, cefaclor, cefdinir, cefditoren, cefepime, cefotaxime, cefpodoxime, cefprozil, ceftaroline, ceftizoxime, ceftriaxone, cefuroxime, doripenem, ertapenem, imipenem, loracarbef, meropenem. See general comment (4).									
A	Penicillin	1 µg oxacillin	≥20	–	–	–	–	–	**(6)** Isolates of pneumococci with oxacillin zone sizes of ≥20 mm are susceptible (MIC ≤0.06 µg/mL) to penicillin. Penicillin and cefotaxime, ceftriaxone, or meropenem MICs should be determined for those isolates with oxacillin zone diameters of ≤19 mm, because zones of ≤19 mm occur with penicillin-resistant, -intermediate, or certain -susceptible strains. For isolates with oxacillin zones ≤19 mm, do not report penicillin as resistant without performing a penicillin MIC test.
A	Penicillin parenteral (nonmeningitis)	–	–	–	–	≤2	4	≥8	**(7)** ***Rx:*** Doses of intravenous penicillin of at least 2 million units every 4 hours in adults with normal renal function (12 million units per day) can be used to treat nonmeningeal pneumococcal infections due to strains with penicillin MICs ≤2 µg/mL. Strains with an intermediate MIC of 4 µg/mL may necessitate penicillin doses of 18–24 million units per day. **(8)** For all isolates other than those from CSF, report interpretations for both meningitis and nonmeningitis.
A	Penicillin parenteral (meningitis)	–	–	–	–	≤0.06	–	≥0.12	**(9)** ***Rx:*** Use of penicillin in meningitis requires therapy with maximum doses of intravenous penicillin (eg, at least 3 million units every 4 hours in adults with normal renal function). **(10)** For CSF isolates, report only meningitis interpretations. See general comment (4).
A	Penicillin (oral penicillin V)	–	–	–	–	≤0.06	0.12–1	≥2	**(11)** Interpretations for oral penicillin may be reported for isolates other than those from CSF.

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Table 2G. *Streptococcus pneumoniae* (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
PENICILLINS (Continued)									
C	Amoxicillin (nonmeningitis)	–	–	–	–	≤2	4	≥8	
C	Amoxicillin-clavulanate (nonmeningitis)					≤2/1	4/2	≥8/4	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.) See comment (5).									
O	Cefepime (meningitis)	–	–	–	–	≤0.5	1	≥2	**(12)** In the United States, for CSF isolates, report only nonmeningitis interpretations. There is not an FDA-approved indication for the use of cefepime for meningitis in the United States.
B	Cefepime (nonmeningitis)	–	–	–	–	≤1	2	≥4	**(13)** In the United States, only report interpretations for nonmeningitis and include the nonmeningitis notation on the report.
B	Cefotaxime (meningitis)	–	–	–	–	≤0.5	1	≥2	**(14)** For CSF isolates, report only meningitis interpretations. **(15)** ***Rx:*** Use of cefotaxime or ceftriaxone in meningitis requires therapy with maximum doses. See general comment (4).
B	Ceftriaxone (meningitis)	–	–	–	–	≤0.5	1	≥2	
B	Cefotaxime (nonmeningitis)	–	–	–	–	≤1	2	≥4	**(16)** For all isolates other than those from CSF, report interpretations for both meningitis and nonmeningitis.
B	Ceftriaxone (nonmeningitis)	–	–	–	–	≤1	2	≥4	
C	Ceftaroline (nonmeningitis)	30 µg	≥26	–	–	≤0.5	–	–	**(17)** Breakpoints are based on a dosage regimen of 600 mg every 12 h.
C	Cefuroxime (parenteral)	–	–	–	–	≤0.5	1	≥2	
CEPHEMS (ORAL) See comment (5).									
C	Cefuroxime (oral)	–	–	–	–	≤1	2	≥4	
O	Cefaclor	–	–	–	–	≤1	2	≥4	
O	Cefdinir	–	–	–	–	≤0.5	1	≥2	
O	Cefpodoxime	–	–	–	–	≤0.5	1	≥2	
O	Cefprozil	–	–	–	–	≤2	4	≥8	
O	Loracarbef	–	–	–	–	≤2	4	≥8	

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Table 2G. *Streptococcus pneumoniae* (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
CARBAPENEMS See comment (5).									
B	Meropenem	–	–	–	–	≤0.25	0.5	≥1	See general comment (4) and comment (6).
C	Ertapenem	–	–	–	–	≤1	2	≥4	
C	Imipenem	–	–	–	–	≤0.12	0.25–0.5	≥1	
O	Doripenem	–	–	–	–	≤1	–	–	
GLYCOPEPTIDES									
B	Vancomycin	30 µg	≥17	–	–	≤1	–	–	See general comment (4).
MACROLIDES **(18)** Susceptibility and resistance to azithromycin, clarithromycin, and dirithromycin can be predicted by testing erythromycin. **(19)** Not routinely reported for organisms isolated from the urinary tract.									
A	Erythromycin	15 µg	≥21	16–20	≤15	≤0.25	0.5	≥1	
O	Azithromycin	15 µg	≥18	14–17	≤13	≤0.5	1	≥2	
O	Clarithromycin	15 µg	≥21	17–20	≤16	≤0.25	0.5	≥1	
O	Dirithromycin	15 µg	≥18	14–17	≤13	≤0.5	1	≥2	
O	Telithromycin	15 µg	≥19	16–18	≤15	≤1	2	≥4	
TETRACYCLINES **(20)** Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline.									
B	Tetracycline	30 µg	≥28	25–27	≤24	≤1	2	≥4	
B	Doxycycline	30 µg	≥28	25–27	≤24	≤0.25	0.5	≥1	
FLUOROQUINOLONES									
B	Gemifloxacin	5 µg	≥23	20–22	≤19	≤0.12	0.25	≥0.5	**(21)** *S. pneumoniae* isolates susceptible to levofloxacin are predictably susceptible to gemifloxacin and moxifloxacin. However, *S. pneumoniae* susceptible to gemifloxacin or moxifloxacin cannot be assumed to be susceptible to levofloxacin.
B	Levofloxacin	5 µg	≥17	14–16	≤13	≤2	4	≥8	
B	Moxifloxacin	5 µg	≥18	15–17	≤14	≤1	2	≥4	
O	Gatifloxacin	5 µg	≥21	18–20	≤17	≤1	2	≥4	
O	Ofloxacin	5 µg	≥16	13–15	≤12	≤2	4	≥8	
O	Sparfloxacin	5 µg	≥19	16–18	≤15	≤0.5	1	≥2	
FOLATE PATHWAY ANTAGONISTS									
A	Trimethoprim-sulfamethoxazole	1.25/23.75 µg	≥19	16–18	≤15	≤0.5/9.5	1/19–2/38	≥4/76	
PHENICOLS									
C	Chloramphenicol	30 µg	≥21	–	≤20	≤4	–	≥8	See comment (19).
ANSAMYCINS									
C	Rifampin	5 µg	≥19	17–18	≤16	≤1	2	≥4	**(22)** ***Rx:*** Rifampin should not be used alone for antimicrobial therapy.

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Table 2G. *Streptococcus pneumoniae* (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
LINCOSAMIDES									
B	Clindamycin	2 µg	≥19	16–18	≤15	≤0.25	0.5	≥1	**(23)** Inducible clindamycin resistance can be detected by disk diffusion using the D-zone test or by broth microdilution using the single-well test (containing both erythromycin and clindamycin) (see Table 3**G**, Subchapter 3.9 in M02,[2] and Subchapter 3.12 in M07[1]). See comment (19).
STREPTOGRAMINS									
O	Quinupristin-dalfopristin	15 µg	≥19	16–18	≤15	≤1	2	≥4	
OXAZOLIDINONES									
C	Linezolid	30 µg	≥21	–	–	≤2	–	–	

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; CSF, cerebrospinal fluid; FDA, US Food and Drug Administration; I, intermediate; LHB, lysed horse blood; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; R, resistant; S, susceptible.

References for Table 2G

1 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

2 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

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Table 2H-1. Zone Diameter and MIC Breakpoints for *Streptococcus* spp. β-Hemolytic Group

Testing Conditions

Medium: Disk diffusion: MHA with 5% sheep blood
Broth dilution: CAMHB with LHB (2.5% to 5% v/v); the CAMHB should be supplemented to 50 µg/mL calcium for daptomycin (see M07[1] for instructions for preparation of LHB)
Agar dilution: MHA with sheep blood (5% v/v); recent studies using the agar dilution method have not been performed and reviewed by the subcommittee.

Inoculum: Colony suspension, equivalent to a 0.5 McFarland standard, using colonies from an overnight (18- to 20-hour) sheep blood agar plate

Incubation: 35°C ± 2°C
Disk diffusion: 5% CO_2; 20–24 hours
Dilution methods: ambient air; 20–24 hours (CO_2 if necessary for growth with agar dilution)

Routine QC Recommendations (see Tables 4B and 5B for acceptable QC ranges)

S. pneumoniae ATCC®* 49619

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

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

Refer to Table **3G** for additional testing recommendations, reporting suggestions, and QC.

General Comments

(1) For disk diffusion, test a maximum of 9 disks on a 150-mm plate and 4 disks on a 100-mm plate. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Do not measure the zone of inhibition of hemolysis. Measure the zones from the upper surface of the agar illuminated with reflected light, with the cover removed. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth.

(2) For β-hemolytic streptococci when testing chloramphenicol, clindamycin, erythromycin, linezolid, tedizolid, and tetracycline by broth microdilution MIC, trailing growth can make end-point determination difficult. In such cases, read the MIC at the lowest concentration where the trailing begins. Tiny buttons of growth should be ignored (see M07,[1] Figures 3 and 4).

(3) For this table, the β-hemolytic group includes the large colony–forming pyogenic strains of streptococci with group A (*S. pyogenes*), C, or G antigens and strains with Group B (*S. agalactiae*) antigen. Small colony–forming β-hemolytic strains with group A, C, F, or G antigens (*S. anginosus* group, previously termed *"S. milleri"*) are considered part of the viridans group, and breakpoints for the viridans group should be used (see Table 2H-2).

(4) Penicillin and ampicillin are drugs of choice for treatment of β-hemolytic streptococcal infections. Susceptibility testing of penicillins and other β-lactams approved by the US Food and Drug Administration for treatment of β-hemolytic streptococcal infections does not need to be performed routinely, because nonsusceptible isolates (ie, penicillin MICs > 0.12 and ampicillin MICs > 0.25 µg/mL) are extremely rare in any β-hemolytic streptococcus and have not been reported for *S. pyogenes*. If testing is performed, any β-hemolytic streptococcal isolate found to be nonsusceptible should be re-identified, retested, and, if confirmed, submitted to a public health laboratory. (See Appendix A for additional instructions.)

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Table 2H-1. *Streptococcus* spp. β-Hemolytic Group (Continued)

(5) Breakpoints for *Streptococcus* spp. β-hemolytic group are proposed based on population distributions of various species, pharmacokinetics of the antimicrobial agents, previously published literature, and the clinical experience of subcommittee members. Systematically collected clinical data were not available for review with many of the antimicrobial agents in this table.

NOTE: Information in boldface type is new or modified since the previous edition.

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
PENICILLINS **(6)** An organism that is susceptible to penicillin can be considered susceptible to antimicrobial agents listed here when used for approved indications and does not need to be tested against those agents. For groups A, B, C, and G β-hemolytic streptococci, penicillin is a surrogate for ampicillin, amoxicillin, amoxicillin-clavulanate, ampicillin-sulbactam, cefazolin, cefepime, ceftaroline, cephradine, cephalothin, cefotaxime, ceftriaxone, ceftizoxime, imipenem, ertapenem, and meropenem. For group A β-hemolytic streptococci, penicillin is also a surrogate for cefaclor, cefdinir, cefprozil, ceftibuten, cefuroxime, and cefpodoxime.									
A	Penicillin or	10 units	≥24	–	–	≤0.12	–	–	See general comment (4).
A	ampicillin	10 µg	≥24	–	–	≤0.25	–	–	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.) See comment (6).									
B	Cefepime or	30 µg	≥24	–	–	≤0.5	–	–	
B	cefotaxime or	30 µg	≥24	–	–	≤0.5	–	–	
B	ceftriaxone	30 µg	≥24	–	–	≤0.5	–	–	
C	Ceftaroline	30 µg	≥26	–	–	≤0.5	–	–	**(7)** Breakpoints are based on a dosage regimen of 600 mg every 12 h.
CARBAPENEMS See comment (6).									
O	Doripenem	–	–	–	–	≤0.12	–	–	
O	Ertapenem	–	–	–	–	≤1	–	–	
O	Meropenem	–	–	–	–	≤0.5	–	–	
GLYCOPEPTIDES									
B	Vancomycin	30 µg	≥17	–	–	≤1	–	–	
LIPOGLYCOPEPTIDES									
C	**Dalbavancin**	**–**	**–**	**–**	**–**	**≤0.25**	**–**	**–**	**(8) For reporting against *S. pyogenes*, *S. agalactiae*, *S. dysgalactiae*, and *S. anginosus* group.**
C	Oritavancin	–	–	–	–	≤0.25	–	–	
C	Telavancin	–	–	–	–	≤0.12	–	–	
LIPOPEPTIDES									
C	Daptomycin	–	–	–	–	≤1	–	–	**(9)** Daptomycin should not be reported for isolates from the respiratory tract.

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Table 2H-1. *Streptococcus* spp. β-Hemolytic Group (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
MACROLIDES **(10)** Susceptibility and resistance to azithromycin, clarithromycin, and dirithromycin can be predicted by testing erythromycin. **(11)** Not routinely reported on isolates from the urinary tract.									
A	Erythromycin	15 µg	≥21	16–20	≤15	≤0.25	0.5	≥1	**(12)** ***Rx:*** Recommendations for intrapartum prophylaxis for group B streptococci are penicillin or ampicillin. Although cefazolin is recommended for penicillin-allergic women at low risk for anaphylaxis, those at high risk for anaphylaxis may receive clindamycin. Group B streptococci are susceptible to ampicillin, penicillin, and cefazolin, but may be resistant to erythromycin and clindamycin. When a group B *Streptococcus* is isolated from a pregnant woman with severe penicillin allergy (high risk for anaphylaxis), erythromycin and clindamycin (including inducible clindamycin resistance) should be tested, and only clindamycin should be reported. See Table 3**G**.
O	Azithromycin	15 µg	≥18	14–17	≤13	≤0.5	1	≥2	
O	Clarithromycin	15 µg	≥21	17–20	≤16	≤0.25	0.5	≥1	
O	Dirithromycin	15 µg	≥18	14–17	≤13	≤0.5	1	≥2	
TETRACYCLINES **(13)** Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline.									
O	Tetracycline	30 µg	≥23	19–22	≤18	≤2	4	≥8	
FLUOROQUINOLONES									
C	Levofloxacin	5 µg	≥17	14–16	≤13	≤2	4	≥8	
O	Gatifloxacin	5 µg	≥21	18–20	≤17	≤1	2	≥4	
O	Grepafloxacin	5 µg	≥19	16–18	≤15	≤0.5	1	≥2	
O	Ofloxacin	5 µg	≥16	13–15	≤12	≤2	4	≥8	
O	Trovafloxacin	10 µg	≥19	16–18	≤15	≤1	2	≥4	
PHENICOLS									
C	Chloramphenicol	30 µg	≥21	18–20	≤17	≤4	8	≥16	See comment (11).
LINCOSAMIDES									
A	Clindamycin	2 µg	≥19	16–18	≤15	≤0.25	0.5	≥1	See comments (11) and (12). **(14)** Inducible clindamycin resistance can be detected by disk diffusion using the D-zone test and broth microdilution. See Table 3**G**, Subchapter 3.9 in M02,[2] and Subchapter 3.12 in M07.[1]

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Table 2H-1. *Streptococcus* spp. β-Hemolytic Group (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
STREPTOGRAMINS									
O	Quinupristin-dalfopristin	15 µg	≥19	16–18	≤15	≤1	2	≥4	**(15)** Report against *S. pyogenes*.
OXAZOLIDINONES									
C	Linezolid	30 µg	≥21	–	–	≤2	–	–	
C	Tedizolid	–	–	–	–	≤0.5	–	–	**(16)** For reporting against *S. pyogenes* and *S. agalactiae* only.

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; I, intermediate; LHB, lysed horse blood; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; R, resistant; S, susceptible.

References for Table 2H-1

1 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

2 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests.* 13th ed. CLSI standard M02. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

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Table 2H-2. Zone Diameter and MIC Breakpoints for *Streptococcus* spp. Viridans Group

Testing Conditions	
Medium:	Disk diffusion: MHA with 5% sheep blood Broth dilution: CAMHB with LHB (2.5% to 5% v/v); the CAMHB should be supplemented to 50 µg/mL calcium for daptomycin (see M07[1] for instructions for preparation of LHB) Agar dilution: MHA with sheep blood (5% v/v); recent studies using the agar dilution method have not been performed and reviewed by the subcommittee.
Inoculum:	Colony suspension, equivalent to a 0.5 McFarland standard using colonies from an overnight (18- to 20-hour) sheep blood agar plate
Incubation:	35°C ± 2°C Disk diffusion: 5% CO_2; 20–24 hours Dilution methods: ambient air; 20–24 hours (CO_2 if necessary for growth with agar dilution)

Routine QC Recommendations (see Tables 4B and 5B for acceptable QC ranges)

S. pneumoniae ATCC®* 49619

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

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

General Comments

(1) For disk diffusion, measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Do not measure the zone of inhibition of hemolysis. Measure the zones from the upper surface of the agar illuminated with reflected light, with the cover removed. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth.

(2) For viridans streptococci when testing chloramphenicol, clindamycin, erythromycin, linezolid, tedizolid, and tetracycline by broth microdilution MIC, trailing growth can make end-point determination difficult. In such cases, read the MIC at the lowest concentration where the trailing begins. Tiny buttons of growth should be ignored (see M07,[1] Figures 3 and 4).

(3) The viridans group of streptococci includes the following five groups, with several species within each group: *mutans* group, *salivarius* group, *bovis* group, *anginosus* group (previously "*S. milleri*" group), and *mitis* group. The *anginosus* group includes small colony–forming β-hemolytic strains with groups A, C, F, and G antigens. For detailed information on the species within the groups, please refer to recent literature.

(4) Breakpoints for *Streptococcus* spp. viridans group are proposed based on population distributions of various species, pharmacokinetics of the antimicrobial agents, previously published literature, and the clinical experience of subcommittee members. Systematically collected clinical data were not available for review with many of the antimicrobial agents in this table.

NOTE: Information in boldface type is new or modified since the previous edition.

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Table 2H-2. *Streptococcus* spp. Viridans Group (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
PENICILLINS									
A	Penicillin	–	–	–	–	≤0.12	0.25–2	≥4	**(5)** Viridans streptococci isolated from normally sterile body sites (eg, CSF, blood, bone) should be tested for penicillin susceptibility using an MIC method. **(6)** A penicillin MIC of ≤0.125 µg/mL is the same as a penicillin MIC of ≤0.12 µg/mL and both should be interpreted as susceptible. Laboratories should report an MIC of ≤0.125 µg/mL as ≤0.12 µg/mL. **(7)** ***Rx:*** Penicillin- or ampicillin-intermediate isolates may necessitate combined therapy with an aminoglycoside for bactericidal action.
A	Ampicillin					≤0.25	0.5–4	≥8	
β-LACTAM COMBINATION AGENTS									
C	Ceftolozane-tazobactam	–	–	–	–	≤8/4	16/4	≥32/4	**(8)** Breakpoints are based on a dosage regimen of 1.5 g every 8 h.
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)									
B	Cefepime	30 µg	≥24	22–23	≤21	≤1	2	≥4	
B	Cefotaxime	30 µg	≥28	26–27	≤25	≤1	2	≥4	
B	Ceftriaxone	30 µg	≥27	25–26	≤24	≤1	2	≥4	
CARBAPENEMS									
O	Doripenem	–	–	–	–	≤1	–	–	
O	Ertapenem	–	–	–	–	≤1	–	–	
O	Meropenem	–	–	–	–	≤0.5	–	–	
GLYCOPEPTIDES									
B	Vancomycin	30 µg	≥17	–	–	≤1	–	–	
LIPOGLYCOPEPTIDES									
C	**Dalbavancin**	**–**	**–**	**–**	**–**	**≤0.25**	**–**	**–**	**(9) For reporting against *S. pyogenes*, *S. agalactiae*, *S. dysgalactiae*, and *S. anginosus* group.**
C	Oritavancin	–	–	–	–	≤0.25	–	–	
C	Telavancin	–	–	–	–	≤0.06	–	–	
LIPOPEPTIDES									
O	Daptomycin	–	–	–	–	≤1	–	–	**(10)** Daptomycin should not be reported for isolates from the respiratory tract.

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Table 2H-2. *Streptococcus* spp. Viridans Group (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
MACROLIDES **(11)** Susceptibility and resistance to azithromycin, clarithromycin, and dirithromycin can be predicted by testing erythromycin. **(12)** Not routinely reported on isolates from the urinary tract.									
C	Erythromycin	15 µg	≥21	16–20	≤15	≤0.25	0.5	≥1	
O	Azithromycin	15 µg	≥18	14–17	≤13	≤0.5	1	≥2	
O	Clarithromycin	15 µg	≥21	17–20	≤16	≤0.25	0.5	≥1	
O	Dirithromycin	15 µg	≥18	14–17	≤13	≤0.5	1	≥2	
TETRACYCLINES **(13)** Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline.									
O	Tetracycline	30 µg	≥23	19–22	≤18	≤2	4	≥8	
FLUOROQUINOLONES									
O	Levofloxacin	5 µg	≥17	14–16	≤13	≤2	4	≥8	
O	Ofloxacin	5 µg	≥16	13–15	≤12	≤2	4	≥8	
O	Gatifloxacin	5 µg	≥21	18–20	≤17	≤1	2	≥4	
O	Grepafloxacin	5 µg	≥19	16–18	≤15	≤0.5	1	≥2	
O	Trovafloxacin	10 µg	≥19	16–18	≤15	≤1	2	≥4	
PHENICOLS									
C	Chloramphenicol	30 µg	≥21	18–20	≤17	≤4	8	≥16	See comment (12).
LINCOSAMIDES									
C	Clindamycin	2 µg	≥19	16–18	≤15	≤0.25	0.5	≥1	See comment (12).
STREPTOGRAMINS									
O	Quinupristin-dalfopristin	15 µg	≥19	16–18	≤15	≤1	2	≥4	
OXAZOLIDINONES									
C	Linezolid	30 µg	≥21	–	–	≤2	–	–	
C	Tedizolid	–	–	–	–	≤0.25	–	–	**(14)** For reporting against *S. anginosus* group (includes *S. anginosus, S. intermedius,* and *S. constellatus*) only.

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; CSF, cerebrospinal fluid; I, intermediate; LHB, lysed horse blood; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; R, resistant; S, susceptible.

Reference for Table 2H-2

[1] CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

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Table 2I. Zone Diameter and MIC Breakpoints for *Neisseria meningitidis*

Testing Conditions

Medium: Disk diffusion: MHA with 5% sheep blood
Broth microdilution: CAMHB supplemented with LHB (2.5% to 5% v/v) (see M07[1] for preparation of LHB)
Agar dilution: MHA supplemented with sheep blood (5% v/v)

Inoculum: Colony suspension from 20–24 hours growth from chocolate agar incubated at 35°C; 5% CO_2; equivalent to a 0.5 McFarland standard. Colonies grown on sheep blood agar may be used for inoculum preparation. However, the 0.5 McFarland suspension obtained from sheep blood agar will contain approximately 50% fewer CFU/mL. This must be taken into account when preparing the final dilution before panel inoculation, as guided by colony counts.

Incubation: 35°C ± 2°C; 5% CO_2; 20–24 hours

Routine QC Recommendations (See Tables 4A-**1**, 4B, 5A-**1**, and 5B for acceptable QC ranges.)

Streptococcus pneumoniae ATCC®* 49619:

Disk diffusion: incubate in 5% CO_2.

Broth microdilution: incubate in ambient air or CO_2 (except azithromycin QC tests that must be incubated in ambient air).

E. coli ATCC® 25922

Disk diffusion, broth microdilution or agar dilution for ciprofloxacin, nalidixic acid, minocycline, and sulfisoxazole: incubate in ambient air or CO_2.

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

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

General Comments

Important: For complete information on safety precautions, see *Biosafety in Microbiological and Biomedical Laboratories.* 5th ed. Washington, DC: US Department of Health and Human Services; 2009. http://www.cdc.gov/biosafety/publications/bmbl5/. Accessed November 20, 2017.

(1) **Recommended precautions:** Perform all AST of *N. meningitidis* in a BSC. Manipulating *N. meningitidis* outside a BSC is associated with increased risk for contracting meningococcal disease. Laboratory-acquired meningococcal disease is associated with a case fatality rate of 50%. Exposure to droplets or aerosols of *N. meningitidis* is the most likely risk for laboratory-acquired infection. Rigorous protection from droplets or aerosols is mandated when microbiological procedures (including AST) are performed on all *N. meningitidis* isolates.

(2) If a BSC is unavailable, manipulation of these isolates should be minimized, limited to Gram staining or serogroup identification using phenolized saline solution, while wearing a laboratory coat and gloves and working behind a full face splash shield. Use BSL-3 practices, procedures, and containment equipment for activities with a high potential for droplet or aerosol production and for activities involving production quantities or high concentrations of infectious materials. If BSL-2 or BSL-3 facilities are not available, forward isolates to a referral or public health laboratory with a minimum of BSL-2 facilities.

(3) Laboratorians who are exposed routinely to potential aerosols of *N. meningitidis* should consider vaccination according to the current recommendations of the Centers for Disease Control and Prevention Advisory Committee on Immunization Practices, available at http://www.cdc.gov/vaccines/acip/index.html.

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Table 2I. *Neisseria meningitidis* (Continued)

(4) For disk diffusion, test a maximum of 5 disks on a 150-mm plate and 2 disks on a 100-mm plate. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Measure the zones from the upper surface of the agar illuminated with reflected light, with the cover removed. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.

(5) Breakpoints are based on population distributions of MICs of various agents, pharmacokinetics of the agents, previously published literature, and the clinical experience of subcommittee members. Systematically collected clinical data were not available to review with many of the antimicrobial agents in this table.

(6) With azithromycin, breakpoints were developed initially using MICs determined by incubation in ambient air for the pharmacodynamic calculations.

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
PENICILLINS									
C	Penicillin		–	–	–	≤0.06	0.12–0.25	≥0.5	
C	Ampicillin		–	–	–	≤0.12	0.25–1	≥2	
CEPHEMS									
C	Cefotaxime or	30 µg	≥34	–	–	≤0.12	–	–	
C	ceftriaxone	30 µg	≥34	–	–	≤0.12	–	–	
CARBAPENEMS									
C	Meropenem	10 µg	≥30	–	–	≤0.25	–	–	
MACROLIDES									
C	Azithromycin	15 µg	≥20	–	–	≤2	–	–	See general comment (6). **(7)** May be appropriate only for prophylaxis of meningococcal case contacts. These breakpoints do not apply to therapy of patients with invasive meningococcal disease.
TETRACYCLINES									
C	Minocycline	30 µg	≥26	–	–	≤2	–	–	See comment (7).
FLUOROQUINOLONES **(8)** For surveillance purposes, a nalidixic acid MIC ≥8 µg/mL or a zone ≤25 mm may correlate with diminished fluoroquinolone susceptibility.									
C	Ciprofloxacin	5 µg	≥35	33–34	≤32	≤0.03	0.06	≥0.12	See comment (7).
C	Levofloxacin	–	–	–	–	≤0.03	0.06	≥0.12	

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Table 2I. *Neisseria meningitidis* (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
FOLATE PATHWAY ANTAGONISTS									
C	Sulfisoxazole	–	–	–	–	≤2	4	≥8	See comment (7).
C	Trimethoprim-sulfamethoxazole	1.25/23.75 µg	≥30	26–29	≤25	≤0.12/2.4	0.25/4.75	≥0.5/9.5	**(9)** Trimethoprim-sulfamethoxazole is the preferred disk for detection of sulfonamide resistance. Trimethoprim-sulfamethoxazole testing predicts susceptibility and resistance to trimethoprim-sulfamethoxazole and sulfonamides. Sulfonamides may be appropriate only for prophylaxis of meningococcal case contacts.
PHENICOLS									
C	Chloramphenicol	30 µg	≥26	20–25	≤19	≤2	4	≥8	**(10)** Not routinely reported on isolates from the urinary tract.
ANSAMYCINS									
C	Rifampin	5 µg	≥25	20–24	≤19	≤0.5	1	≥2	See comment (7).

Abbreviations: AST, antimicrobial susceptibility testing; ATCC®, American Type Culture Collection; BSC, biological safety cabinet; BSL-2, biosafety level 2; BSL-3, biosafety level 3; CAMHB, cation-adjusted Mueller-Hinton broth; CFU, colony-forming unit(s); I, intermediate; LHB, lysed horse blood; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; R, resistant; S, susceptible.

Reference for Table 2I

1 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

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Table 2J. MIC Breakpoints for Anaerobes

Testing Conditions

Medium: Agar dilution (for all anaerobes): Brucella agar supplemented with hemin (5 μg/mL), Vitamin K_1 (1 μg/mL), and laked sheep blood (5% v/v)
Broth microdilution (for *B. fragilis* group only): Brucella broth supplemented with hemin (5 μg/mL), Vitamin K_1 (1 μg/mL), and LHB (5% v/v)

Inoculum: **Broth culture** method or colony suspension, equivalent to 0.5 McFarland suspension;
Agar: 10^5 CFU per spot
Broth: 10^6 CFU/mL

Incubation: 36°C ± 1°C, anaerobically
Broth microdilution: 46–48 hours
Agar dilution: 42–48 hours

Routine QC Recommendations (see Tables 5D and 5E for acceptable QC ranges)

Test one or more of the following organisms. The choice and number of QC strains tested should be based on obtaining on-scale end points for the antimicrobial agent tested.

B. fragilis ATCC®* 25285
Bacteroides thetaiotaomicron ATCC® 29741
Clostridioides* (formerly *Clostridium*) *difficile ATCC® 700057
Eggerthella lenta (formerly *Eubacterium lentum*) ATCC® 43055

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

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

General Comments

(1) For isolates for which the antimicrobial agent MICs fall within the intermediate category, maximum dosages, along with proper ancillary therapy, should be used to achieve the best possible levels of drug in abscesses and/or poorly perfused tissues. If this approach is taken, organisms for which the antimicrobial agent MICs fall within the susceptible range are generally amenable to therapy. Organisms for which the antimicrobial agent MICs are in the intermediate range may respond, but in such cases efficacy as measured by patient clinical response should be carefully monitored. Ancillary therapy, such as drainage procedures and debridement, are of great importance for proper management of anaerobic infections.

(2) Refer to Figures 3 and 4 in CLSI document M11[1] for examples of reading end points.

(3) MIC values using either Brucella blood agar or Wilkins Chalgren agar (former reference medium) are considered equivalent.

(4) Broth microdilution is only recommended for testing the *B. fragilis* group. MIC values for agar or broth microdilution are considered equivalent for that group.

(5) Until additional studies are performed to validate broth microdilution for testing other organisms, it should be used only for testing members of the *B. fragilis* group.

NOTE: Information in boldface type is new or modified since the previous edition.

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Table 2J. Anaerobes (Continued)

Test/Report Group	Antimicrobial Agent	Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	
PENICILLINS					
A/C	Ampicillin[a]	≤0.5	1	≥2	**(6)** Ampicillin and penicillin are recommended for primary testing and reporting for gram-positive organisms (group A) because most of them are β-lactamase negative, but not for gram-negative organisms (group C) because many are β-lactamase positive. **(7)** Members of the *B. fragilis* group are presumed to be resistant. Other gram-negative and gram-positive anaerobes may be screened for β-lactamase activity with a chromogenic cephalosporin; if β-lactamase positive, report as resistant to penicillin, ampicillin, and amoxicillin. Be aware that β-lactamase-negative isolates may be resistant to β-lactams by other mechanisms. Because higher blood levels are achievable with these antimicrobial agents, infection with non–β-lactamase-producing organisms with higher MICs (2–4 µg/mL) with adequate dosage regimen might be treatable. **(8)** Results of ampicillin testing can be used to predict results for amoxicillin.
A/C	Penicillin[a]	≤0.5	1	≥2	
O	Piperacillin	≤32	64	≥128	
β-LACTAM COMBINATION AGENTS					
A	Amoxicillin-clavulanate	≤4/2	8/4	≥16/8	
A	Ampicillin-sulbactam	≤8/4	16/8	≥32/16	
A	Piperacillin-tazobactam	≤**16**/4	**32/4–** 64/4	≥128/4	
O	Ticarcillin-clavulanate	≤32/2	64/2	≥128/2	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)					
C	Cefotetan	≤16	32	≥64	
C	Cefoxitin	≤16	32	≥64	
C	Ceftizoxime	≤32	64	≥128	
C	Ceftriaxone	≤16	32	≥64	
O	Cefmetazole	≤16	32	≥64	
O	Cefoperazone	≤16	32	≥64	
O	Cefotaxime	≤16	32	≥64	
CARBAPENEMS					
A	Doripenem	≤2	4	≥8	
A	Ertapenem	≤4	8	≥16	
A	Imipenem	≤4	8	≥16	
A	Meropenem	≤4	8	≥16	
TETRACYCLINES					
C	Tetracycline	≤4	8	≥16	
FLUOROQUINOLONES					
C	Moxifloxacin	≤2	4	≥8	

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Table 2J. Anaerobes (Continued)

Test/Report Group	Antimicrobial Agent	Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	
LINCOSAMIDES					
A	Clindamycin	≤2	4	≥8	
PHENICOLS					
C	Chloramphenicol	≤8	16	≥32	
NITROIMIDAZOLES					
A	Metronidazole	≤8	16	≥32	**(9)** Many non–spore-forming, gram-positive anaerobic rods are resistant to metronidazole.

Abbreviations: ATCC®, American Type Culture Collection; CFU, colony-forming unit(s); I, intermediate; LHB, lysed horse blood; MIC, minimal inhibitory concentration; QC, quality control; R, resistant; S, susceptible.

Footnote

a. A/C: Group A for gram-positive organisms and group C for *B. fragilis* and other gram-negative organisms. Refer to Table 1C.

Reference for Table 2J

1 CLSI. *Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard—Eighth Edition*. CLSI document M11-A8. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.

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M100, 28th ed.

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Table 3A. Tests for Extended-Spectrum β-Lactamases in *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Escherichia coli,* and *Proteus mirabilis*

NOTE: Following evaluation of PK-PD properties, limited clinical data, and MIC distributions, revised breakpoints for cefazolin, cefotaxime, ceftazidime, ceftizoxime, ceftriaxone, and aztreonam were published in January 2010 (M100-S20) and are listed in Table 2A. Cefuroxime (parenteral) was also evaluated; however, no change in breakpoints was necessary with the dosage. When using the current breakpoints, routine ESBL testing is no longer necessary before reporting results (ie, it is no longer necessary to edit results for cephalosporins, aztreonam, or penicillins to resistant). However, ESBL testing may still be useful for epidemiological or infection control purposes. For laboratories that have not implemented the current breakpoints, ESBL testing should be performed as described in this table.

Note that breakpoints for drugs with limited availability in many countries (eg, moxalactam, cefonicid, cefamandole, and cefoperazone) were not evaluated. If considering use of these drugs for *E. coli, Klebsiella* spp., or *Proteus* spp., ESBL testing should be performed. If isolates test ESBL positive, the results for moxalactam, cefonicid, cefamandole, and cefoperazone should be reported as resistant.

Test	Criteria for Performance of ESBL Test		ESBL Test	
Test method	Disk diffusion	Broth microdilution	Disk diffusion	Broth microdilution
Medium	MHA	CAMHB	MHA	CAMHB
Antimicrobial concentration	For *K. pneumoniae, K. oxytoca,* and *E. coli*: Cefpodoxime 10 μg or Ceftazidime 30 μg or Aztreonam 30 μg or Cefotaxime 30 μg or Ceftriaxone 30 μg For *P. mirabilis*: Cefpodoxime 10 μg or Ceftazidime 30 μg or Cefotaxime 30 μg (Using more than one antimicrobial agent improves the sensitivity of ESBL detection.)	For *K. pneumoniae, K. oxytoca,* and *E. coli*: Cefpodoxime 4 μg/mL or Ceftazidime 1 μg/mL or Aztreonam 1 μg/mL or Cefotaxime 1 μg/mL or Ceftriaxone 1 μg/mL For *P. mirabilis*: Cefpodoxime 1 μg/mL or Ceftazidime 1 μg/mL or Cefotaxime 1 μg/mL (Using more than one antimicrobial agent improves the sensitivity of ESBL detection.)	Ceftazidime 30 μg Ceftazidime-clavulanate[a] 30/10 μg and Cefotaxime 30 μg Cefotaxime-clavulanate 30/10 μg (Testing necessitates using both cefotaxime and ceftazidime, alone and in combination with clavulanate.)	Ceftazidime 0.25–128 μg/mL Ceftazidime-clavulanate 0.25/4–128/4 μg/mL and Cefotaxime 0.25–64 μg/mL Cefotaxime-clavulanate 0.25/4–64/4 μg/mL (Testing necessitates using both cefotaxime and ceftazidime, alone and in combination with clavulanate.)
Inoculum	Standard disk diffusion procedure	Standard broth dilution procedure	Standard disk diffusion procedure	Standard broth dilution procedure
Incubation conditions	35°C±2°C; ambient air	35°C±2°C; ambient air	35°C±2°C; ambient air	35°C±2°C; ambient air
Incubation length	16–18 hours	16–20 hours	16–18 hours	16–20 hours

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Table 3A. (Continued)

Test	Criteria for Performance of ESBL Test		ESBL Test	
Test method	Disk diffusion	Broth microdilution	Disk diffusion	Broth microdilution
Results	For *K. pneumoniae, K. oxytoca,* and *E. coli*: Cefpodoxime zone ≤17 mm Ceftazidime zone ≤22 mm Aztreonam zone ≤27 mm Cefotaxime zone ≤27 mm Ceftriaxone zone ≤25 mm For *P. mirabilis*: Cefpodoxime zone ≤22 mm Ceftazidime zone ≤22 mm Cefotaxime zone ≤27 mm Zones above may indicate ESBL production.	Growth at or above the concentrations listed may indicate ESBL production (ie, for *E. coli, K. pneumoniae,* and *K. oxytoca,* MIC ≥8 μg/mL for cefpodoxime or MIC ≥2 μg/mL for ceftazidime, aztreonam, cefotaxime, or ceftriaxone; and for *P. mirabilis,* MIC ≥2 μg/mL for cefpodoxime, ceftazidime, or cefotaxime).	A ≥5-mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanate vs the zone diameter of the agent when tested alone = ESBL (eg, ceftazidime zone = 16; ceftazidime-clavulanate zone = 21).	A ≥3 twofold concentration decrease in an MIC for either antimicrobial agent tested in combination with clavulanate vs the MIC of the agent when tested alone = ESBL (eg, ceftazidime MIC = 8 μg/mL; ceftazidime-clavulanate MIC = 1 μg/mL).
Reporting			For all confirmed ESBL-producing strains: If laboratories do not use current cephalosporin and aztreonam breakpoints, the test interpretation should be reported as resistant for all penicillins, cephalosporins, and aztreonam. If laboratories use current cephalosporin and aztreonam breakpoints, then test interpretations for these agents do not need to be changed from susceptible to resistant.	

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Table 3A. (Continued)

Test	Criteria for Performance of ESBL Test		ESBL Test	
Test method	Disk diffusion	Broth microdilution	Disk diffusion	Broth microdilution
QC recommendations	When testing antimicrobial agents used for ESBL detection, *K. pneumoniae* ATCC®[b] 700603 is provided as a supplemental QC strain (eg, for training, competence assessment, or test evaluation). Either strain, *K. pneumoniae* ATCC® 700603 or *E. coli* ATCC® 25922, may then be used for routine QC (eg, weekly or daily). *E. coli* ATCC® 25922 (see acceptable QC ranges in Table 4A-**1**) *K. pneumoniae* ATCC® 700603: Cefpodoxime zone 9–16 mm Ceftazidime zone 10–18 mm Aztreonam zone 9–17 mm Cefotaxime zone 17–25 mm Ceftriaxone zone 16–24 mm	When testing antimicrobial agents used for ESBL detection, *K. pneumoniae* ATCC® 700603 is provided as a supplemental QC strain (eg, for training, competence assessment, or test evaluation). Either strain, *K. pneumoniae* ATCC® 700603 or *E. coli* ATCC® 25922, may then be used for routine QC (eg, weekly or daily). *E. coli* ATCC® 25922 = No growth (see acceptable QC ranges listed in Table 5A-**1**) *K. pneumoniae* ATCC® 700603 = Growth: Cefpodoxime MIC ≥ 8 μg/mL Ceftazidime MIC ≥ 2 μg/mL Aztreonam MIC ≥ 2 μg/mL Cefotaxime MIC ≥ 2 μg/mL Ceftriaxone MIC ≥ 2 μg/mL	When performing the ESBL test, *K. pneumoniae* ATCC® 700603 and *E. coli* ATCC® 25922 should be used for routine QC (eg, weekly or daily). **Acceptable QC:** *E. coli* ATCC® 25922: ≤ 2-mm increase in zone diameter for antimicrobial agent tested in combination with clavulanate vs the zone diameter when tested alone. *K. pneumoniae* ATCC® 700603: ≥ 5-mm increase in zone diameter of ceftazidime-clavulanate vs ceftazidime alone; ≥ 3-mm increase in zone diameter of cefotaxime-clavulanate vs cefotaxime alone.	When performing the ESBL test, *K. pneumoniae* ATCC® 700603 and *E. coli* ATCC® 25922 should be tested routinely (eg, weekly or daily). **Acceptable QC:** *E. coli* ATCC® 25922: < 3 twofold concentration decrease in MIC for antimicrobial agent tested in combination with clavulanate vs the MIC of the agent when tested alone. *K. pneumoniae* ATCC® 700603: ≥ 3 twofold concentration decrease in MIC for an antimicrobial agent tested in combination with clavulanate vs the MIC of the agent when tested alone.

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; ESBL, extended-spectrum β-lactamase; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; PK-PD, pharmacokinetic-pharmacodynamic; QC, quality control.

Footnotes

a. Preparation of ceftazidime-clavulanate (30 μg/10 μg) and cefotaxime-clavulanate (30 μg/10 μg) disks: Using a stock solution of clavulanate at 1000 μg/mL (either freshly prepared or taken from small aliquots that have been frozen at −70°C), add 10 μL of clavulanate to ceftazidime (30 μg) and cefotaxime (30 μg) disks. Use a micropipette to apply the 10 μL of stock solution to the ceftazidime and cefotaxime disks within one hour before they are applied to the plates, allowing about 30 minutes for the clavulanate to absorb and the disks to be dry enough for application. Use disks immediately after preparation or discard; do not store.

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Introduction to Tables 3B and 3C. Tests for Carbapenemases in *Enterobacteriaceae* and *Pseudomonas aeruginosa*

Institutional infection control procedures or epidemiological investigations may necessitate identification of carbapenemase-producing *Enterobacteriaceae* **and** *P. aeruginosa*. Such testing is not currently recommended for routine use.

Carbapenemase-producing isolates of *Enterobacteriaceae* usually test intermediate or resistant to one or more carbapenems using the current breakpoints as listed in Table 2A (**NOTE:** Ertapenem nonsusceptibility is the most sensitive indicator of carbapenemase production) and usually test resistant to one or more agents in cephalosporin subclass III (eg, cefoperazone, cefotaxime, ceftazidime, ceftizoxime, and ceftriaxone). However, some isolates that produce carbapenemases such as SME or IMI often test susceptible to these cephalosporins.

Laboratories using *Enterobacteriaceae* MIC breakpoints for carbapenems described in M100-S20 (January 2010) should perform mCIM **with or without eCIM**, the CarbaNP test, and/or a molecular assay as described below when isolates of *Enterobacteriaceae* are suspicious for carbapenemase production based on imipenem or meropenem MICs of 2–4 µg/mL or ertapenem MIC of 2 µg/mL. Refer to Table 3B-1 for specific steps to use with breakpoints for carbapenems listed in M100-S20 (January 2010).

NOTE: Information in boldface type is new or modified since the previous edition.

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Introduction to Tables 3B and 3C. (Continued)

	Tests Used for Epidemiological or Infection Control–Related Testing			
	CarbaNP (Table 3B)	**mCIM (Table 3C)**	**mCIM with eCIM (Table 3C)**	**Other (eg, molecular assays)**
Organisms	*Enterobacteriaceae* and *P. aeruginosa* that are not susceptible to one or more carbapenems	*Enterobacteriaceae* **and *P. aeruginosa*** that are not susceptible to one or more carbapenems	***Enterobacteriaceae* that are positive by mCIM**	*Enterobacteriaceae* and *P. aeruginosa* that are not susceptible to one or more carbapenems to determine the presence of a carbapenemase, or to determine carbapenemase type in isolates positive by CarbaNP **or mCIM.**
Strengths	Rapid	No special reagents or media necessary	**No special reagents or media necessary**	Determines type of carbapenemase in addition to absence or presence of the enzyme
Limitations	Special reagents are needed, some of which necessitate in-house preparation (and have a short shelf life). Invalid results occur with some isolates. Certain carbapenemase types (eg, OXA-type, chromosomally encoded) are not consistently detected.	Requires overnight incubation	**Requires overnight incubation**	Special reagents and equipment are needed. Specific to targeted genes; false-negative result if specific carbapenemase gene present is not targeted.

Abbreviations: **eCIM, EDTA-modified carbapenem inactivation method**; mCIM, modified carbapenem inactivation method, MIC, minimal inhibitory concentration.

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Table 3B. CarbaNP Test for Suspected Carbapenemase Production in *Enterobacteriaceae* and *Pseudomonas aeruginosa*[1-7]

NOTE: If using FORMER MIC breakpoints for carbapenems described in M100-S20 (January 2010), please refer to modifications in Table 3**B**-1 below.

NOTE: Information in boldface type is new or modified since the previous edition.

Test	CarbaNP Test
When to do this test:	For epidemiological or infection control purposes. **NOTE:** No change in the interpretation of carbapenem susceptibility test results is necessary for CarbaNP–positive isolates. Such testing is not currently recommended for routine use.
Test method	Colorimetric microtube assay
Test reagents and materials	• Clinical laboratory reagent water • Imipenem reference standard powder • Commercially available bacterial protein extraction reagent in Tris HCl buffer, pH 7.4 • Zinc sulfate heptahydrate • Phenol red powder • 1 N NaOH solution • 10% HCl solution • Microcentrifuge tubes 1.5 mL, clear • 1-µL inoculation loops • Containers to store prepared solutions Use reagents above to prepare the following solutions (instructions for preparation are provided below this table): • 10 mM zinc sulfate heptahydrate solution • 0.5% phenol red solution • 0.1 N sodium hydroxide solution • CarbaNP Solution A • CarbaNP Solution B (solution A + imipenem)
Test procedure	1. Label two microcentrifuge tubes (one “a” and one “b”) for each patient isolate, QC organism, and uninoculated reagent control. 2. Add 100 µL of bacterial protein extraction reagent to each tube. 3. For each isolate to be tested, emulsify a 1-µL loopful of bacteria from an overnight blood agar plate in both tubes “a” and “b.” Vortex each tube for 5 seconds. (Uninoculated reagent control tubes should contain only bacterial protein extraction reagent, no organism.) **NOTE:** Do not use growth from selective media or plates containing antibiotics or other agents that select for certain bacteria. 4. Add 100 µL of solution A to tube “a.” 5. Add 100 µL of solution B to tube “b.” 6. Vortex tubes well. 7. Incubate at 35°C ± 2°C for up to 2 hours. Isolates that demonstrate positive results before 2 hours can be reported as carbapenemase producers.

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Table 3B. (Continued)

Test	CarbaNP Test
Test interpretation	Strategy for reading (see Figure 1, below): 1. Read uninoculated reagent control tubes "a" and "b" (ie, "blanks"). • Both tubes must be red or red-orange. • If either tube is any other color, the test is invalid. 2. Read inoculated tube "a." • Tube "a" must be red or red-orange. • If tube "a" is any other color, the test is invalid. 3. Read inoculated tube "b." • Red or red-orange = negative • Light orange, dark yellow, or yellow = positive • Orange = invalid 4. Interpret results as follows:

Results for Patient and QC Tubes		
Tube "a": Solution A (serves as internal control)	**Tube "b": Solution B**	**Interpretation**
Red or red-orange	Red or red-orange	Negative, no carbapenemase detected
Red or red-orange	Light orange, dark yellow, or yellow	Positive, carbapenemase producer
Red or red-orange	Orange	Invalid
Orange, light orange, dark yellow, or yellow	Any color	Invalid

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Table 3B. (Continued)

Test	CarbaNP Test
	NOTES: A slight color change may be observed with the addition of imipenem to solution A. Compare patient tubes to the uninoculated reagent control tubes when interpreting questionable results. For invalid results: • Check reagents for QC strains and uninoculated reagent controls. Reagent deterioration can cause invalid results. An invalid result for an uninoculated reagent control test indicates a problem with solution A and/or solution B. Check the pH of solution A. If pH is < 7.8, prepare fresh solution A and solution B. • Repeat the test, including the uninoculated reagent controls. • If the repeat test is invalid, perform molecular assay.
Reporting	Report positive as "Carbapenemase producer." Report negative as "No carbapenemase detected."
QC recommendations	Test positive and negative QC strains and uninoculated reagent control tubes each day of testing. *K. pneumoniae* ATCC®[a] BAA-1705™—Carbapenemase positive *K. pneumoniae* ATCC® BAA-1706™—Carbapenemase negative Results for uninoculated reagent control tubes "a" and "b" must be negative (ie, red or red-orange). Any other result invalidates all tests performed on that day with the same lot of reagents. The addition of imipenem to tube "b" might cause tube "b" to appear red-orange when tube "a" is red.

Abbreviations: ATCC®, American Type Culture Collection; MIC, minimal inhibitory concentration; QC, quality control.

NOTE 1: Test recommendations were largely derived following testing of US isolates of *Enterobacteriaceae* **and** *P. aeruginosa* and provide for a high level of sensitivity (> 90%) and specificity (> 90%) in detecting *Klebsiella pneumoniae* carbapenemase (KPC), New Delhi metallo-β-lactamase, VIM, IMP, SPM, and SME-type carbapenemases in these isolates. The sensitivity and specificity of the test for detecting other carbapenemase production can vary. For example, the sensitivity of the CarbaNP test for detecting OXA-48-type carbapenemases is low (ie, 11%).

NOTE 2: In CLSI studies, two KPC-positive strains with low carbapenem MICs (one *E. cloacae* susceptible by MIC to all three carbapenems and one *E. coli* that was susceptible to meropenem and intermediate to imipenem and ertapenem) were not detected by this test.

NOTE 3: Additional investigations of CarbaNP with *Acinetobacter* spp. showed poor sensitivity (ie, 21.3% for *A. baumannii*); therefore, the previous recommendation for use of CarbaNP with *Acinetobacter* spp. was removed.

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Table 3B. (Continued)

Footnote

a. ATCC® is a registered trademark of the American Type Culture Collection. Per ATCC® convention, the trademark symbol is used after "BAA" in each catalog number, in conjunction with the registered ATCC® name.

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Table 3B-1. Modifications of Table 3B When Using MIC Breakpoints for Carbapenems Described in M100-S20 (January 2010)[1-5]

Test	CarbaNP Test
When to do this test:	Until laboratories can implement the revised carbapenem MIC breakpoints, this test (or an alternative confirmatory test for carbapenemases) should be performed when isolates of *Enterobacteriaceae* are suspicious for carbapenemase production based on imipenem or meropenem MICs of 2–4 µg/mL or ertapenem MIC of 2 µg/mL.
Reporting	For isolates that are CarbaNP positive, report all carbapenems as resistant, regardless of MIC. If the CarbaNP test is negative, interpret the carbapenem MICs using CLSI breakpoints as listed in Table 2A in M100-S20 (January 2010). **NOTE:** Not all carbapenemase-producing isolates of *Enterobacteriaceae* are CarbaNP positive.

Abbreviation: MIC, minimal inhibitory concentration.

Tables 3B and 3B-1 – Instructions for Preparation of Test Components

The steps for preparing 10 mM zinc sulfate heptahydrate solution are listed below.

Step	Action	Comment
1.	Weigh out 1.4 g of $ZnSO_4 \cdot 7H2O$.	
2.	Add the powder to 500 mL clinical laboratory reagent water.	
3.	Mix the solution.	
4.	Store the solution at room temperature.	Expiration is 1 year or not to exceed expiration of individual components

The steps for preparing 0.5% phenol red solution are listed below.

Step	Action	Comment
1.	Weigh out 1.25 g of phenol red powder.	
2.	Add the powder to 250 mL clinical laboratory reagent water.	
3.	Mix the solution.	
4.	Store the solution at room temperature.	Expiration is 1 year or not to exceed expiration of individual components. **NOTE:** This solution does not remain in solution. Mix well before use.

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Tables 3B and 3B-1. (Continued)

The steps for preparing 0.1 N sodium hydroxide solution are listed below.

Step	Action	Comments
1.	Add 20 mL of 1 N NaOH to 180 mL clinical laboratory reagent water.	
2.	Store the solution at room temperature.	Expiration is 1 year or not to exceed expiration of individual components

The steps for preparing CarbaNP solution A are listed below.

Step	Action	Comments
1.	To a 25- to 50-mL beaker, add 2 mL of 0.5% phenol red solution to 16.6 mL clinical laboratory reagent water.	
2.	Add 180 µL of 10 mM zinc sulfate solution.	
3.	Adjust the pH to 7.8 ± 0.1 with 0.1 N NaOH solution (or 10% HCl solution if pH is too high).	10% HCl solution can be used if the pH is too high.
4.	Store the solution at 4 to 8°C in a small vial or bottle.	Protect the solution from prolonged light exposure. Expiration is 2 weeks or not to exceed expiration of individual components (solution should remain red or red-orange; do not use if solution turns any other color).

The steps for preparing CarbaNP solution B (solution A + 6 mg/mL imipenem) are listed below.

Step	Action	Comment
1.	Determine the amount of solution B needed, allowing 100 µL per tube for each patient, QC strain, and uninoculated reagent control.	**Example:** To test 2 patient isolates, positive and negative controls and an uninoculated reagent control, 500 µL of solution B is needed.
2.	Weigh out approximately 10–20 mg of imipenem powder.	It is advisable to weigh out at least 10 mg of powder. Divide the actual weight by 6 to determine the amount (in mL) of solution A to add to the powder. **Example:** 18 mg of imipenem / 6 = 3 mL of solution A, which is sufficient for 30 tubes.
3.	Store the solution at 4 to 8°C for up to 3 days.	

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Tables 3B and 3B-1. (Continued)

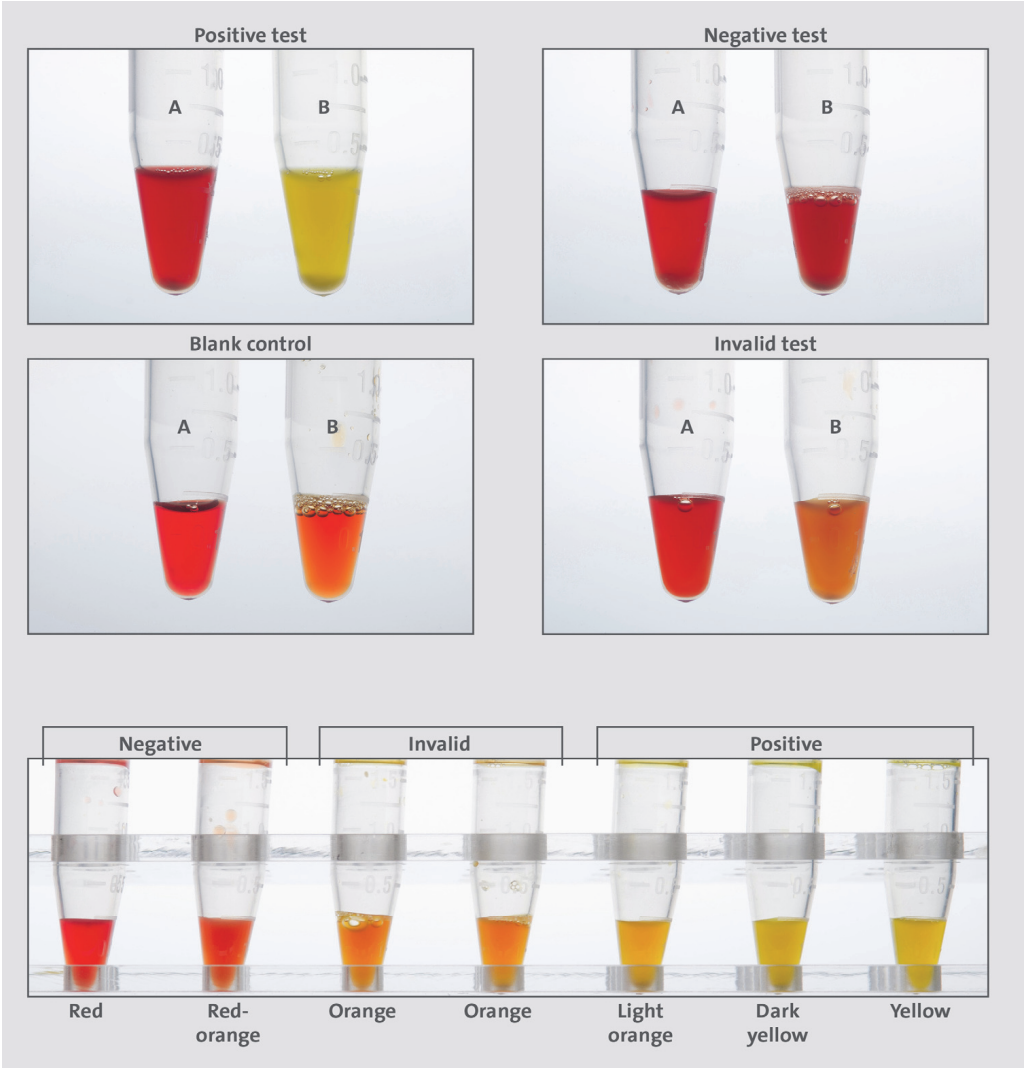

Figure 1. Interpretation of Color Reactions

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Tables 3B and 3B-1. (Continued)

References for Tables 3B and 3B-1

1 Nordmann P, Poirel L, Dortet L. Rapid detection of carbapenemase-producing *Enterobacteriaceae. Emerg Infect Dis.* 2012;18(9):1503-1507.

2 Dortet L, Poirel L, Nordmann P. Rapid detection of carbapenemase-producing *Pseudomonas spp. J Clin Microbiol.* 2012;50(11):3773-3776.

3 Dortet L, Poirel L, Nordmann P. Rapid identification of carbapenemase types in *Enterobacteriaceae* and *Pseudomonas* spp. by using a biochemical test. *Antimicrob Agents Chemother.* 2012;56(12):6437-6440.

4 Cunningham SA, Noorie T, Meunier D, Woodford N, Patel R. Rapid and simultaneous detection of genes encoding *Klebsiella pneumoniae* carbapenemase (bla_{KPC}) and New Delhi metallo-β-lactamase (bla_{NDM}) in Gram-negative bacilli. *J Clin Microbiol.* 2013;51(4):1269-1271.

5 Vasoo S, Cunningham SA, Kohner PC, et al. Comparison of a novel, rapid chromogenic biochemical assay, the Carba NP test, with the modified Hodge test for detection of carbapenemase-producing Gram-negative bacilli. *J Clin Microbiol.* 2013;51(9):3097-3101.

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Table 3C. Modified Carbapenem Inactivation Methods for Suspected Carbapenemase Production in *Enterobacteriaceae* and *P. aeruginosa*[1-4]

NOTE: If using FORMER MIC breakpoints for carbapenems described in M100-S20 (January 2010), please refer to modifications in Table 3**C**-1 below.

Test	mCIM Only or in Conjunction With eCIM
When to do this test:	For epidemiological or infection control purposes. **NOTE:** No change in the interpretation of carbapenem susceptibility test results is necessary for mCIM **positive and/or eCIM results**. mCIM **with or without eCIM** testing is not currently recommended for routine use. • **mCIM is used for detecting carbapenemases in *Enterobacteriaceae* and *P. aeruginosa* whereas eCIM is used together with mCIM to differentiate metallo-β-lactamases from serine carbapenemases in *Enterobacteriaceae*.** • **mCIM can be performed alone; however, eCIM must be performed together with mCIM.** • **eCIM is only valid if mCIM is positive.**
Test method	Meropenem disk inactivation
Test reagents and materials	• TSB (2 mL aliquots) • Meropenem disks (10 µg) • 1-µL and 10-µL inoculation loops • Nutrient broth (eg, Mueller-Hinton, TSB) or normal saline (3.0–5.0 mL aliquots) • MHA plates (100 mm or 150 mm) • Meropenem-susceptible indicator strain – *E. coli* (ATCC®[a] 25922) • **0.5 M EDTA (only for eCIM)**

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Table 3C. (Continued)

Test	mCIM Only or in Conjunction With eCIM
Test procedure: mCIM	1. For each isolate to be tested, emulsify a 1-µL loopful of bacteria **for *Enterobacteriaceae* or 10-µL loopful of bacteria for *P. aeruginosa*** from an overnight blood agar plate in 2 mL TSB. 2. Vortex for 10–15 seconds. 3. Add a 10-µg meropenem disk to each tube using sterile forceps or a single disk dispenser. Ensure the entire disk is immersed in the suspension. 4. Incubate at 35°C ± 2°C in ambient air for 4 hours ± 15 minutes. 5. Just before or immediately following completion of the TSB-meropenem disk suspension incubation, prepare a 0.5 McFarland suspension (using the colony suspension method) of *E. coli* ATCC® 25922 in nutrient broth or saline. 6. Inoculate an MHA plate with *E. coli* ATCC® 25922 as for the routine disk diffusion procedure (see M02[4]) making sure the inoculum suspension preparation and MHA plate inoculation steps are each completed within 15 minutes. Allow the plates to dry for 3–10 minutes before adding the meropenem disks. 7. Remove the meropenem disk from each TSB-meropenem disk suspension using a 10-µL loop by placing the flat side of the loop against the flat edge of the disk and using surface tension to pull the disk out of the liquid. Carefully drag and press the loop along the inside edge of the tube to expel excess liquid from the disk. Continue using the loop to remove the disk from the tube and then place it on the MHA plate previously inoculated with the meropenem-susceptible *E. coli* ATCC® 25922 indicator strain. Disk capacity: 4 disks on a 100 mm MHA plate; 8 disks on a 150 mm MHA plate (see Figure 1). 8. Invert and incubate the MHA plates at 35°C ± 2°C in ambient air for 18–24 hours. 9. Following incubation, measure the zones of inhibition as for the routine disk diffusion method (see M02[4]).
Test procedure: eCIM for *Enterobacteriaceae* only; optional	**1. For each isolate, label a second 2-mL TSB tube for the eCIM test.** **2. Add 20 µL of the 0.5 M EDTA to the 2-mL TSB tube to obtain a final concentration of 5 mM EDTA.** **3. Follow steps 1 through 9 above as for mCIM procedure. Process the mCIM and eCIM tubes in parallel.** **4. Place the meropenem disks from the mCIM and eCIM tubes on the same MHA plate inoculated with the meropenem-susceptible *E.coli* ATCC® 25922 indicator strain.** **NOTE: Additional QC is needed for the eCIM test (see QC recommendations).**

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Table 3C. (Continued)

Test	mCIM Only or in Conjunction With eCIM
Test interpretation	**For additional explanations, refer to Figures 2A, 2B, and 3A through 3D, as well as the notes section below.** **mCIM** • **Carbapenemase positive (see Figures 2A and 2B):** – **Zone diameter of 6–15 mm or presence of pinpoint colonies within a 16–18 mm zone** – **If the test isolate produces a carbapenemase, the meropenem in the disk will be hydrolyzed and there will be no inhibition or limited growth inhibition of the meropenem-susceptible *E. coli* ATCC® 25922.** • **Carbapenemase negative (see Figure 2A):** – **Zone diameter of ≥19 mm (clear zone)** – **If the test isolate does not produce carbapenemase, the meropenem in the disk will not be hydrolyzed and will inhibit growth of the meropenem-susceptible *E. coli* ATCC® 25922.** • **Carbapenemase indeterminate:** – **Zone diameter of 16–18 mm** – **Zone diameter of ≥19 mm and the presence of pinpoint colonies within the zone** – **The presence or absence of a carbapenemase cannot be confirmed.** **eCIM – Interpret only when mCIM test is positive** • **Metallo-β-lactamase positive:** – **A ≥5-mm increase in zone diameter for eCIM vs zone diameter for mCIM (eg, mCIM = 6 mm; eCIM = 15 mm; zone diameter difference = 9 mm). For only the eCIM test, ignore pinpoint colonies within any zone of inhibition (see Figures 3B and 3C).** – **If the test isolate produces a metallo-β-lactamase, the activity of the carbapenemase will be inhibited in the presence of EDTA such that the meropenem in the disk will not be hydrolyzed as efficiently as in the tube without EDTA. The result is inhibition of the meropenem-susceptible *E. coli* and an increase in the zone diameter for the eCIM zone diameter when compared to the mCIM zone diameter.** • **Metallo-β-lactamase negative:** – **A ≤4-mm increase in zone diameter for the eCIM vs zone diameter of mCIM (eg, mCIM = 6 mm; eCIM = 8 mm; zone diameter difference = 2 mm). For only the eCIM test, ignore pinpoint colonies within any zone of inhibition (see Figure 3D).** – **If the test isolate produces a serine carbapenemase, the activity of the carbapenemase will not be affected by the presence of EDTA and there will be no or marginal (≤4 mm) increase in zone diameter in the presence of EDTA compared to the mCIM zone diameter.**

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Table 3C. (Continued)

<table>
<tr><th>Test</th><th>mCIM Only or in Conjunction With eCIM</th></tr>
<tr><td>Reporting</td><td>
<table>
<tr><th colspan="3">mCIM Only</th></tr>
<tr><th>mCIM Result</th><th>eCIM Result</th><th>Report</th></tr>
<tr><td>Negative</td><td>Not set up</td><td>Carbapenemase not detected</td></tr>
<tr><td>Positive</td><td>Not set up</td><td>Carbapenemase detected</td></tr>
<tr><td>Indeterminate</td><td>Not set up</td><td>Testing inconclusive for the presence of carbapenemase. Call laboratory to discuss.*</td></tr>
<tr><th colspan="3">mCIM and eCIM Combination Test</th></tr>
<tr><th>mCIM Result</th><th>eCIM Result</th><th>Report</th></tr>
<tr><td>Negative</td><td>Do not interpret</td><td>Carbapenemase not detected</td></tr>
<tr><td>Positive</td><td>Negative</td><td>Serine carbapenemase detected</td></tr>
<tr><td>Positive</td><td>Positive</td><td>Metallo-β-lactamase detected</td></tr>
<tr><td>Indeterminate</td><td>Do not interpret</td><td>Testing inconclusive for the presence of carbapenemase. Call laboratory to discuss.*</td></tr>
</table>
* If indeterminate results are obtained on repeat testing, consider performing a different phenotypic test for carbapenemase detection (ie, CarbaNP), a test for carbapenemase genes or send isolate to a referral laboratory for further testing.

If both a serine carbapenemase and a metallo-β-lactamase are co-produced by one organism, differentiation between enzymes will not be possible and false-negative eCIM results may occur.
</td></tr>
</table>

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Table 3C. (Continued)

Test	mCIM Only or in Conjunction With eCIM
NOTES	• For **mCIM** indeterminate results: – Check test isolate and *E. coli* ATCC® 25922 indicator strain for purity. – Check meropenem disk integrity by confirming acceptable results were obtained when disks were subjected to routine disk diffusion test QC. – Repeat the mCIM **and/or eCIM** for test isolate and QC strains. • **mCIM only: For** some **tests, pinpoint** colonies **of the indicator organism (*E. coli* ATCC® 25922) may be observed** within the zone **of inhibition**. If **the colonies are present within a 6- to 18-mm** zone **of inhibition, the test should be considered carbapenemase positive. If colonies are present within a** ≥19-mm **zone, the test should be considered** indeterminant. • **eCIM only: Ignore pinpoint colonies within any zone of inhibition. Interpret results strictly based on the difference in zone diameters between the mCIM and eCIM tests.** • **mCIM negative and eCIM positive results should not occur. If this happens, perform checks as indicated in the first bullet above. If the repeat tests are the same, consider the tests invalid.** • **CLSI has currently standardized mCIM for *Enterobacteriaceae* with a 1-µL loopful of bacteria and *P. aeruginosa* 10-µL loopful of bacteria only.**
QC recommendations	Test positive and negative QC strains each day of testing (refer to Figures 2A and 2B for examples of positive and negative QC results). **QC Strain** / **Organism Characteristic** / **Expected Result** ***K. pneumoniae* ATCC® BAA-1705™** / **KPC positive, Serine carbapenemase producer** / **mCIM positive, eCIM negative** ***K. pneumoniae* ATCC® BAA-1706™** / **Carbapenemase negative** / **mCIM negative** ***K. pneumoniae* ATCC® BAA-2146™*** / **NDM positive, Metallo-β-lactamase producer** / **mCIM positive, eCIM positive** * **eCIM positive control; to be set up only when the eCIM test is performed.** In addition, perform QC of meropenem disks and test media daily or weekly following the routine disk diffusion QC procedure, and handle disks as described in M02.[4] Alternatively, perform QC of meropenem disks with each run by removing a disk from the cartridge of disks used for the run and placing it on the MHA plate inoculated with *E. coli* ATCC® 25922; incubate as above.

Abbreviations: ATCC®, American Type Culture Collection; **eCIM, EDTA-modified carbapenem inactivation method**; **EDTA, ethylenediaminetetraacetic acid;** mCIM, modified carbapenem inactivation method; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; **NDM, New Delhi metallo-β-lactamase;** QC, quality control; TSB, trypticase soy broth.

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Table 3C. (Continued)

NOTE 1: mCIM: This method demonstrated a sensitivity > 99% and specificity > 99% for detection of KPC, NDM, VIM, IMP, IMI, SPM, SME and OXA-type carbapenemases among *Enterobacteriaceae* isolates investigated by CLSI.[b] This method demonstrated a sensitivity > 97% and specificity 100% for detection of KPC, NDM, VIM, IMP, IMI, SPM and OXA-type carbapenemases among *P. aeruginosa* isolates investigated by CLSI.[b] Performance for other carbapenemases or for testing isolates of non-*Enterobacteriaceae* other than *P. aeruginosa* has not been established. Investigations of mCIM with *Acinetobacter* spp. showed poor specificity and poor reproducibility between laboratories and performing mCIM with *Acinetobacter* spp. is not endorsed by CLSI.

In CLSI studies, one OXA-232-producing *K. pneumoniae* isolate **was negative by this assay at** 4 of 9 **validation sites.**

NOTE 2: eCIM: This method demonstrated a sensitivity > 95% and specificity > 92% for differentiation of metallo-β-lactamases (NDM, VIM, and IMP) from serine carbapenemases (KPC, OXA, and SME) among *Enterobacteriaceae* isolates investigated by CLSI.[b] In CLSI studies, one *K. pneumoniae* co-producing NDM and OXA-181 yielded a false-negative result at 3 out of 4 validation sites.

NOTE 3: Information in boldface type is new or modified since the previous edition.

Footnotes

a. ATCC® is a registered trademark of the American Type Culture Collection. Per ATCC® convention, the trademark symbol is used after "BAA" in each catalog number, in conjunction with the registered ATCC® name.

b. The AR Isolate Bank (http://www.cdc.gov/drugresistance/resistance-bank/overview.html) is a centralized repository of microbial pathogens with well-characterized resistance profiles that are assembled by the Centers for Disease Control in collaboration with the US Food and Drug Administration.

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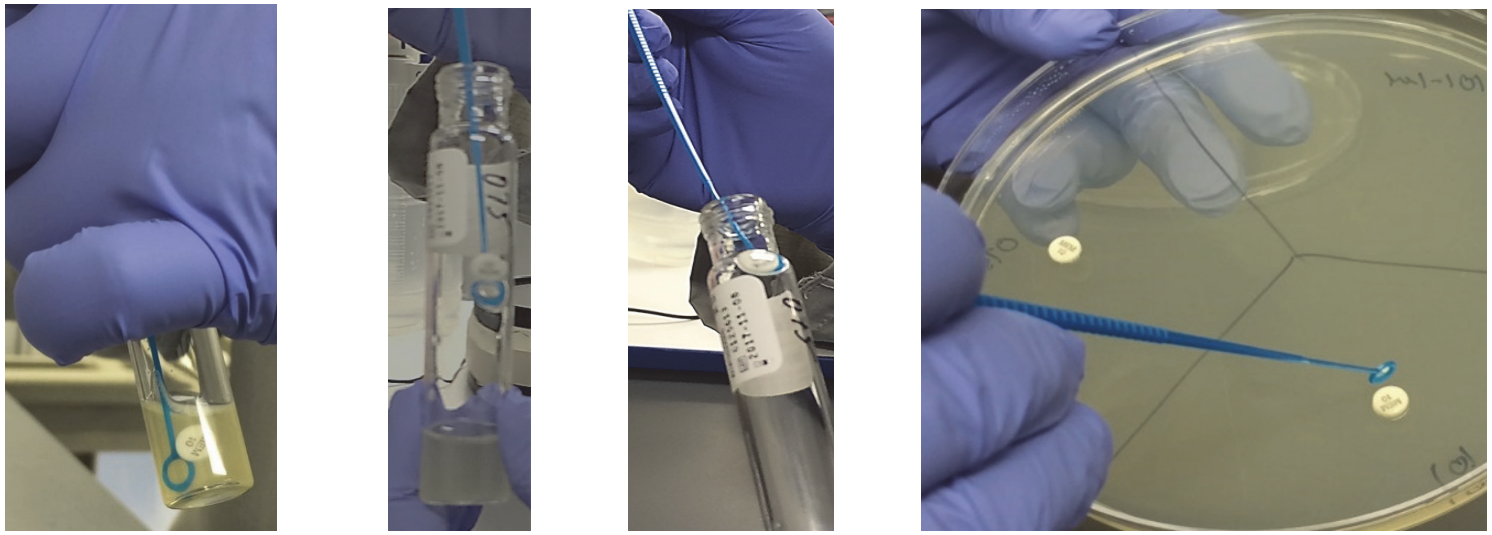
Table 3C. (Continued)



A B C D

Figure 1. Procedure for Placing Meropenem Disks for the mCIM. Remove the meropenem disk with a 10-µL loop (A), and drag the loop against the inside edge of the tube to expel any excess liquid (B). Use the same loop to remove the disk from the tube (C), and place it on the MHA plate (D) previously inoculated with the meropenem-susceptible *E. coli* (ATCC® 25922) indicator strain.

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Table 3C. (Continued)

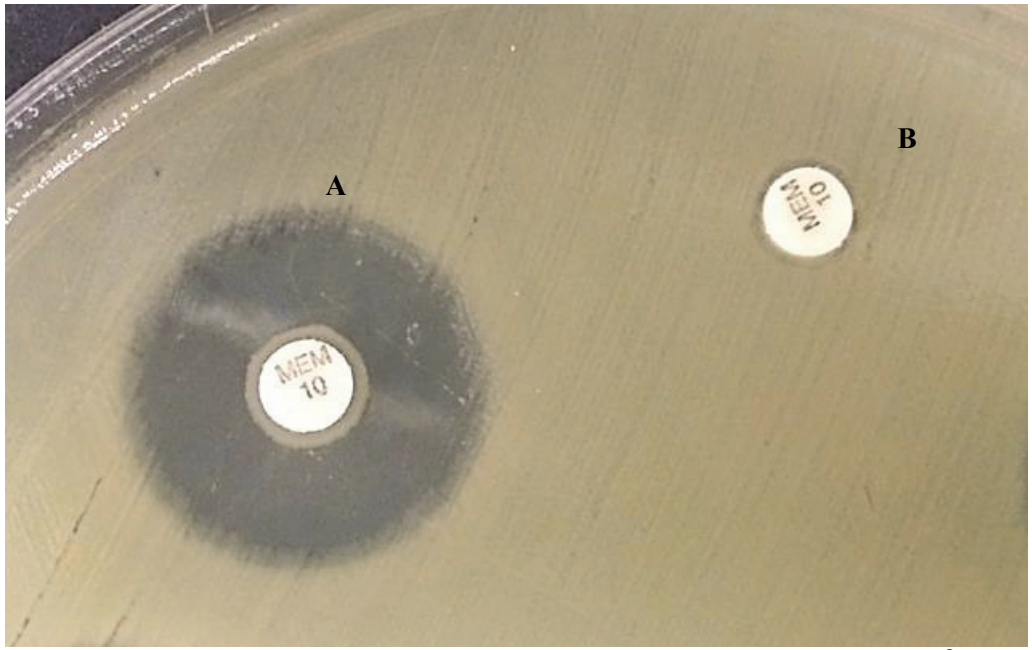

Figure 2A. mCIM Results for QC Strains: Negative Control *K. pneumoniae* ATCC® BAA-1706™ (A) and Positive Control *K. pneumoniae* ATCC® BAA-1705™ (B). NOTE: A narrow ring of growth around the meropenem disk as seen with the negative control (A) results from carryover of the test organism in the TSB and should be ignored.

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Table 3C. (Continued)

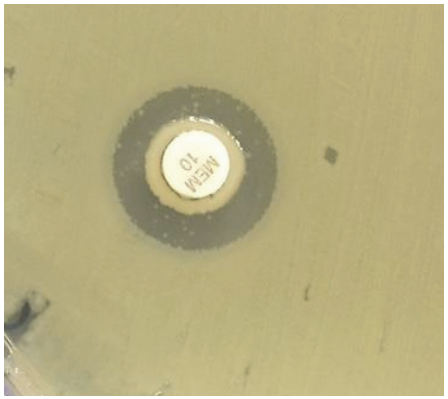

Figure 2B. mCIM Test Interpretation

- **Result: positive mCIM**
- **Report: carbapenemase detected**

NOTE: A narrow ring of growth around the meropenem disk results from carryover of the test organism in the TSB and should be ignored.

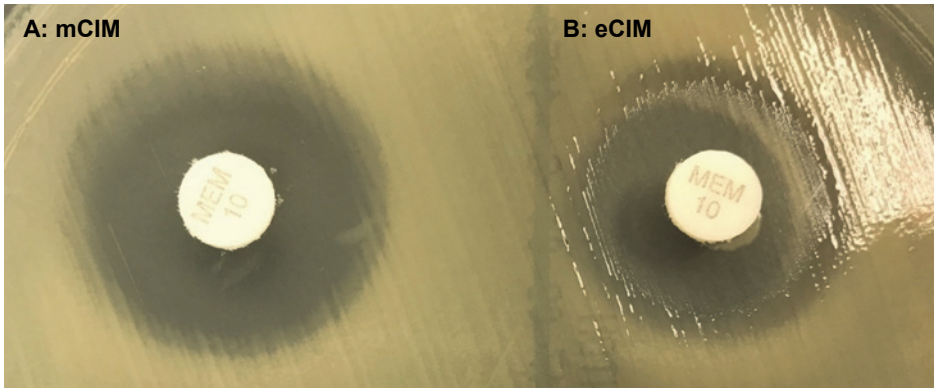

Figure 3A. mCIM and eCIM Test Interpretation: Negative mCIM. "A" shows an mCIM negative result (zone diameter = 20 mm) and "B" shows an eCIM invalid result. Do not interpret the eCIM result when the mCIM is negative as the isolate is negative for carbapenemase production.

- **Result: negative for carbapenemase production**
- **Report: carbapenemase not detected**

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Table 3C. (Continued)

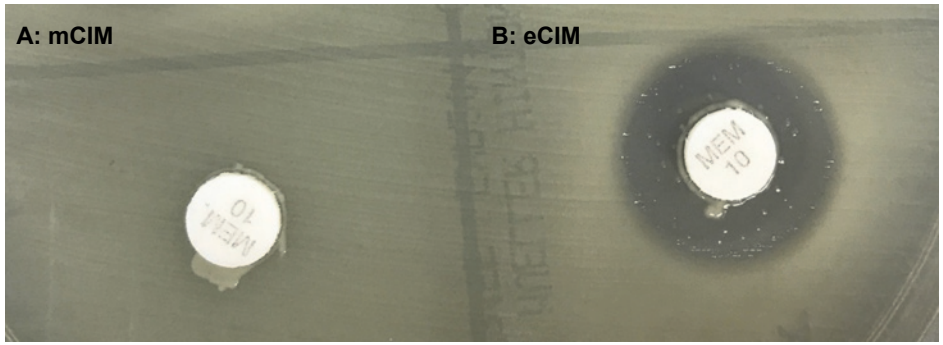

Figure 3B. mCIM and eCIM Test Interpretation: Positive mCIM and eCIM. "A" shows an mCIM positive result (zone diameter of 6 mm) and "B" shows an eCIM positive result (zone diameter = 15 mm with pinpoint colonies throughout the zone of inhibition). NOTE: The pinpoint colonies throughout the zone of inhibition are ignored when measuring the zone for the eCIM test. A ≥ 5-mm increase in zone diameter for eCIM vs zone diameter for mCIM (15 mm − 6 mm = 9 mm) demonstrates the inhibition of the metallo-β-lactamase in the presence of EDTA.

- **Result: positive mCIM and eCIM**
- **Report: metallo-β-lactamase detected**

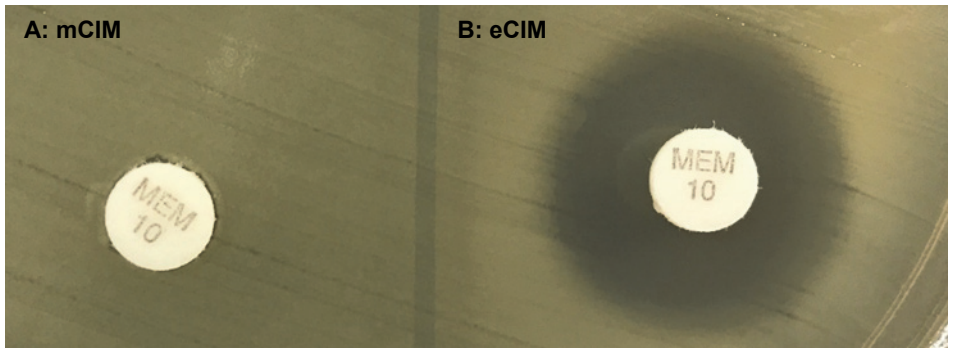

Figure 3C. mCIM and eCIM Test Interpretation: Positive mCIM and eCIM. "A" shows an mCIM positive result (zone diameter = 6 mm) and "B" shows an eCIM positive result (zone diameter = 19 mm). A ≥ 5-mm increase in zone diameter for eCIM vs diameter for mCIM zone (19 mm − 6 mm = 13 mm) demonstrates the inhibition of the metallo-β-lactamase in the presence of EDTA.

- **Result: positive mCIM and eCIM**
- **Report: metallo-β-lactamase detected**

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Table 3C. (Continued)

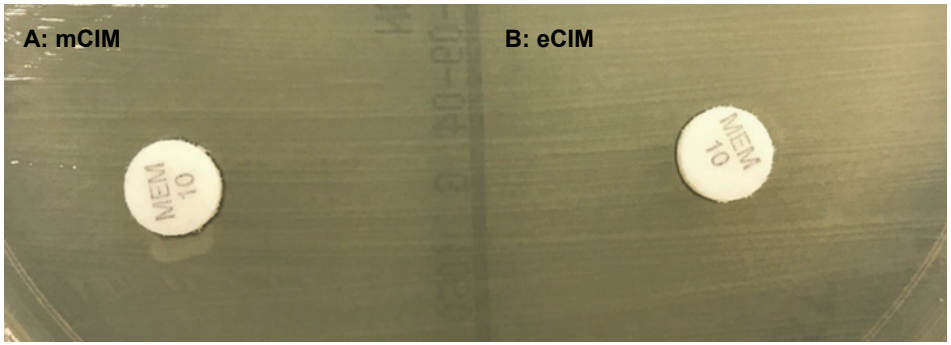

Figure 3D. mCIM and eCIM Test Interpretation: Positive mCIM and Negative eCIM. "A" shows an mCIM positive result (zone diameter = 6 mm) and "B" shows an eCIM negative result (zone diameter = 6 mm). Serine carbapenemases are not inhibited by EDTA and demonstrate a ≤ 4-mm increase in zone diameter for eCIM vs zone diameter for mCIM.

- **Result: positive mCIM and negative eCIM**
- **Report: serine carbapenemase detected**

References for Table 3C

1 Tijet N, Patel SN, Melano RG. Detection of carbapenemase activity in *Enterobacteriaceae*: comparison of the carbapenem inactivation method versus the Carba NP test. *J Antimicrob Chemother*. 2016;71(1):274-276.

2 van der Zwaluw K, de Haan A, Pluister GN, Bootsma HJ, de Neeling AJ, Schouls LM. The carbapenem inactivation method (CIM), a simple and low-cost alternative for the Carba NP test to assess phenotypic carbapenemase activity in gram-negative rods. *PLoS One*. 2015;10(3):e0123690.

3 **Pierce VM, Simner PJ, Lonsway DR, et al. Modified carbapenem inactivation method (mCIM) for phenotypic detection of carbapenemase production among *Enterobacteriaceae*. *J Clin Microbiol*. 2017;55(8): 2321-2333.**

4 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests.* 13th ed. CLSI standard M02. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

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Table 3C-1. Modifications of Table 3C When Using MIC Breakpoints for Carbapenems Described in M100-S20 (January 2010)

Test	mCIM
When to do this test:	Until laboratories can implement the revised carbapenem MIC breakpoints, this test (or an alternative confirmatory test for carbapenemases) should be performed when isolates of *Enterobacteriaceae* are suspicious for carbapenemase production based on imipenem or meropenem MICs of 2–4 µg/mL or ertapenem MIC of 2 µg/mL.
Reporting	For isolates that are mCIM positive, report all carbapenems as resistant, regardless of MIC. If the mCIM test is negative, interpret the carbapenem MICs using CLSI breakpoints as listed in Table 2A in M100-S20 (January 2010). **NOTE:** Not all carbapenemase-producing isolates of *Enterobacteriaceae* are mCIM positive.

Abbreviations: mCIM, modified carbapenem inactivation method; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration.

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Table 3D. Test for Detection of β-Lactamase Production in *Staphylococcus* spp.

Test	β-Lactamase Production	
Test method	**Disk Diffusion (penicillin zone-edge test)**	**Nitrocefin-based test**
Organism group	*S. aureus* with penicillin MICs ≤ 0.12 µg/mL or zones ≥ 29 mm[a]	*S. aureus*[a] and CoNS (including *S. lugdunensis*[b]) with penicillin MICs ≤ 0.12 µg/mL or zones ≥ 29 mm
Medium	MHA	N/A
Antimicrobial concentration	10 units penicillin disk	N/A
Inoculum	Standard disk diffusion procedure	Induced growth (ie, growth taken from the zone margin surrounding a penicillin or cefoxitin disk test on either MHA or a blood agar plate after 16–18 hours of incubation)
Incubation conditions	35°C ± 2°C; ambient air	Room temperature
Incubation length	16–18 hours	Up to 1 hour for nitrocefin-based test or follow manufacturer's directions
Results	Sharp zone edge ("cliff") = β-lactamase positive (see Figure 1 below this table) Fuzzy zone edge ("beach") = β-lactamase negative (see Figure 2 below this table)	Nitrocefin-based test: conversion from yellow to red/pink = β-lactamase positive.
Additional testing and reporting	β-lactamase-positive staphylococci are resistant to penicillin, amino-, carboxy-, and ureidopenicillins.	Nitrocefin-based tests can be used for *S. aureus,* but negative results should be confirmed with the penicillin zone-edge test before reporting penicillin as susceptible. β-lactamase-positive staphylococci are resistant to penicillin, amino-, carboxy-, and ureidopenicillins.
QC recommendations – routine[c]	*S. aureus* ATCC®[d] 25923 for routine QC of penicillin disk to include examination of zone edge test (fuzzy edge = "beach")	
QC recommendations – lot/shipment[e]		*S. aureus* ATCC® 29213 – positive *S. aureus* ATCC® 25923 – negative (or see local regulations and manufacturers' recommendations)
QC recommendations – supplemental[f]	*S. aureus* ATCC® 29213 – positive penicillin zone-edge test (sharp edge = "cliff")	

Abbreviations: ATCC®, American Type Culture Collection; CoNS, coagulase-negative staphylococci; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control.

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Table 3D. (Continued)

Footnotes

a. The penicillin disk diffusion zone-edge test was shown to be more sensitive than nitrocefin-based tests for detection of β-lactamase production in *S. aureus.* The penicillin zone-edge test is recommended if only one test is used for β-lactamase detection. However, some laboratories may choose to perform a nitrocefin-based test first and, if this test is positive, report the results as positive for β-lactamase (or penicillin resistant). If the nitrocefin test is negative, the penicillin zone-edge test should be performed before reporting the isolate as penicillin susceptible in cases in which penicillin may be used for therapy (eg, endocarditis).[1,2]

b. For *S. lugdunensis,* tests for β-lactamase detection are not necessary because isolates producing a β-lactamase will test penicillin resistant (MIC > 0.12 µg/mL and zone diameters < 29 mm). If a laboratory is using a method other than the CLSI disk diffusion or MIC reference method and is unsure if the method can reliably detect penicillin resistance with contemporary isolates of *S. lugdunensis,* the laboratory should perform an induced nitrocefin assay or other CLSI reference method on isolates that test penicillin susceptible before reporting the isolate as penicillin susceptible.

c. QC recommendations – routine

Test negative (susceptible) QC strain:

- With each new lot/shipment of testing materials
- Weekly if the test is performed at least once a week and criteria for converting from daily to weekly QC testing have been met (see Subchapter 4.7.2.3 in M02[3] and M07[4])
- Daily if the test is performed less than once per week and/or if criteria for converting from daily to weekly QC testing have not been met

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

e. QC recommendations – lot/shipment

Test positive (resistant) QC strain at minimum with each new lot/shipment of testing materials.

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Table 3D. (Continued)

f. QC recommendations – supplemental

- Supplemental QC strains can be used to assess a new test, for training personnel, and for competence assessment. It is not necessary to include supplemental QC strains in routine daily or weekly antimicrobial susceptibility testing QC programs. See Appendix C, which describes use of QC strains.

Figure 1. Positive Penicillin Disk Zone-Edge Test for β-Lactamase Detection. The zone edge is sharp or like a "cliff" indicating β-lactamase production.

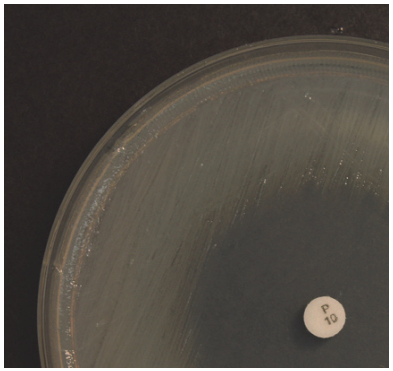

Figure 2. Negative Penicillin Disk Zone-Edge Test for β-Lactamase Detection. The zone edge is fuzzy or like a "beach" indicating no β-lactamase production.

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Table 3D. (Continued)

References for Table 3D

1 Kaase M, Lenga S, Friedrich S, et al. Comparison of phenotypic methods for penicillinase detection in *Staphylococcus aureus*. *Clin Microbiol Infect.* 2008;14(6):614-616.

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

3 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

4 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

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Table 3E. Test for Detection of Methicillin Resistance (Oxacillin Resistance) in *Staphylococcus* spp., Except *Staphylococcus pseudintermedius* and *Staphylococcus schleiferi*

Test	Oxacillin Resistance	*mecA*-Mediated Oxacillin Resistance Using Cefoxitin		
Test method	**Agar Dilution**	**Disk Diffusion**		**Broth Microdilution**
Organism group	*S. aureus*	*S. aureus* and *S. lugdunensis*	CoNS[a]	*S. aureus* and *S. lugdunensis*
Medium	MHA with 4% NaCl	MHA		CAMHB
Antimicrobial concentration	6 μg/mL oxacillin	30 μg cefoxitin disk		4 μg/mL cefoxitin
Inoculum	Colony suspension to obtain 0.5 McFarland turbidity Using a 1-μL loop that was dipped in the suspension, spot an area 10–15 mm in diameter. Alternatively, using a swab dipped in the suspension and the excess liquid expressed, spot a similar area or streak an entire quadrant.	Standard disk diffusion procedure		Standard broth microdilution procedure
Incubation conditions	33 to 35°C; ambient air (Testing at temperatures above 35°C may not detect MRSA.)	33 to 35°C; ambient air (Testing at temperatures above 35°C may not detect MRSA.)		33 to 35°C; ambient air (Testing at temperatures above 35°C may not detect MRSA.)
Incubation length	24 hours; read with transmitted light	16–18 hours	24 hours (may be reported after 18 hours, if resistant)	16–20 hours
Results	Examine carefully with transmitted light for > 1 colony or light film of growth. > 1 colony = oxacillin resistant	≤ 21 mm = *mecA* positive ≥ 22 mm = *mecA* negative	≤ 24 mm = *mecA* positive ≥ 25 mm = *mecA* negative	> 4 μg/mL = *mecA* positive ≤ 4 μg/mL = *mecA* negative
Additional testing and reporting	Oxacillin-resistant staphylococci are resistant to all β-lactam agents; other β-lactam agents should be reported as resistant or should not be reported.	Cefoxitin is used as a surrogate for *mecA*-mediated oxacillin resistance. Isolates that test as *mecA* positive should be reported as oxacillin (not cefoxitin) resistant; other β-lactam agents, except those with anti-MRSA activity, should be reported as resistant or should not be reported.		Cefoxitin is used as a surrogate for *mecA*-mediated oxacillin resistance. Isolates that test as *mecA* positive should be reported as oxacillin (not cefoxitin) resistant; routine testing of other β-lactam agents, except those with anti-MRSA activity, is not advised. Because of the rare occurrence of oxacillin resistance mechanisms other than *mecA,* isolates that test as *mecA* negative, but for which the oxacillin MICs are resistant (MIC ≥ 4 μg/mL), should be reported as oxacillin resistant.

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Table 3E. (Continued)

Test	Oxacillin Resistance	*mecA*-Mediated Oxacillin Resistance Using Cefoxitin		
Test method	**Agar Dilution**	**Disk Diffusion**		**Broth Microdilution**
Organism group	*S. aureus*	*S. aureus* and *S. lugdunensis*	CoNS[a]	*S. aureus* and *S. lugdunensis*
QC recommendations – routine[b]	*S. aureus* ATCC®[c] 29213 – susceptible (with each test day)	*S. aureus* ATCC® 25923 – *mecA* negative (cefoxitin zone 23–29 mm)		*S. aureus* ATCC® 29213 – *mecA* negative (cefoxitin MIC 1–4 µg/mL)
QC recommendations – lot/shipment[d]	*S. aureus* ATCC® 43300 – resistant	*S. aureus* ATCC® 43300 – *mecA* positive (zone ≤21 mm)		*S. aureus* ATCC® 43300 – *mecA* positive (MIC >4 µg/mL)

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; CoNS, coagulase-negative staphylococci; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; MRSA, methicillin-resistant *S. aureus;* QC, quality control.

Footnotes

a. Except *S. lugdunensis,* which is included in the *S. aureus* group.

b. QC recommendations – routine

Test negative (susceptible) QC strain:

- With each new lot/shipment of testing materials
- Weekly if the test is performed at least once a week and criteria for converting from daily to weekly QC testing have been met (see Subchapter 4.7.2.3 in M02[1] and M07[2])
- Daily if the test is performed less than once per week and/or if criteria for converting from daily to weekly QC testing have not been met

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

d. QC recommendations – lot/shipment

Test positive (resistant) QC strain at minimum with each new lot/shipment of testing materials.

NOTE: Information in boldface type is new or modified since the previous edition.

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Table 3E. (Continued)

References for Table 3E

1 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

2 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

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Table 3F. Vancomycin Agar Screen for *Staphylococcus aureus* and *Enterococcus* spp.

Screen Test	Vancomycin MIC ≥8 μg/mL	
Test method	**Agar Dilution**	**Agar Dilution**
Organism group	*S. aureus*	*Enterococcus* spp.
Medium	BHI agar	BHI[a] agar
Antimicrobial concentration	6 μg/mL vancomycin	6 μg/mL vancomycin
Inoculum	Colony suspension to obtain 0.5 McFarland turbidity Preferably, using a micropipette, spot a 10-μL drop onto agar surface. Alternatively, using a swab dipped in the suspension and the excess liquid expressed, spot an area 10–15 mm in diameter or streak a portion of the plate.	1–10 μL of a 0.5 McFarland suspension spotted onto agar surface. Alternatively, using a swab dipped in the suspension and the excess liquid expressed, spot an area 10–15 mm in diameter or streak a portion of the plate.
Incubation conditions	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air
Incubation length	24 hours	24 hours
Results	Examine carefully with transmitted light for > 1 colony or light film of growth. > 1 colony = Presumptive reduced susceptibility to vancomycin	> 1 colony = Presumptive vancomycin resistance
Additional testing and reporting	Perform a vancomycin MIC using a validated MIC method to determine vancomycin MICs on *S. aureus* that grow on BHI–vancomycin screening agar. Testing on BHI–vancomycin screening agar does not reliably detect all vancomycin-intermediate *S. aureus* strains. Some strains for which the vancomycin MICs are 4 μg/mL will fail to grow.	Perform vancomycin MIC on *Enterococcus* spp. that grow on BHI–vancomycin screening agar and test for motility and pigment production to distinguish species with acquired resistance (eg, *vanA* and *vanB*) from those with intrinsic, intermediate-level resistance to vancomycin (eg, *vanC*), such as *Enterococcus gallinarum* and *Enterococcus casseliflavus,* which often grow on the vancomycin screen plate. In contrast to other enterococci, *E. casseliflavus* and *E. gallinarum* with vancomycin MICs of 8–16 μg/mL (intermediate) differ from vancomycin-resistant enterococcus for infection control purposes.
QC recommendations – routine[b]	*E. faecalis* ATCC®[c] 29212 – susceptible	*E. faecalis* ATCC® 29212 – susceptible
QC recommendations – lot/shipment[d]	*E. faecalis* ATCC® 51299 – resistant	*E. faecalis* ATCC® 51299 – resistant

Abbreviations: ATCC®, American Type Culture Collection; BHI, brain heart infusion; MIC, minimal inhibitory concentration; QC, quality control.

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Table 3F. (Continued)

Footnotes

a. BHI: even though not as widely available, dextrose phosphate agar and broth have been shown in limited testing to perform comparably.

b. QC recommendations – routine

Test negative (susceptible) QC strain:

- With each new lot/shipment of testing materials
- Weekly if the test is performed at least once a week and criteria for converting from daily to weekly QC testing have been met (see Subchapter 4.7.2.3 in M02[1] and M07[2])
- Daily if the test is performed less than once per week and/or if criteria for converting from daily to weekly QC testing have not been met

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

d. QC recommendations – lot/shipment

Test positive (resistant) QC strain at minimum with each new lot/shipment of testing materials.

References for Table 3F

1 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

2 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

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Table 3G. Test for Detection of Inducible Clindamycin Resistance in *Staphylococcus* spp., *Streptococcus pneumoniae,* and *Streptococcus* spp. β-Hemolytic Group[a]

Test	Inducible Clindamycin Resistance			
Test method	**Disk Diffusion (D-zone test)**		**Broth Microdilution**	
Organism group (applies only to organisms resistant to erythromycin and susceptible or intermediate to clindamycin)	*S. aureus, S. lugdunensis,* and CoNS	*S. pneumoniae* and β-hemolytic *Streptococcus* spp.	*S. aureus, S. lugdunensis,* and CoNS[b]	*S. pneumoniae* and β-hemolytic *Streptococcus* spp.
Medium	MHA or blood agar purity plate used with MIC tests	MHA supplemented with sheep blood (5% v/v) or TSA supplemented with sheep blood (5% v/v)	CAMHB	CAMHB with LHB (2.5% to 5% v/v)
Antimicrobial concentration	15-µg erythromycin and 2-µg clindamycin disks spaced 15–26 mm apart	15-µg erythromycin and 2-µg clindamycin disks spaced 12 mm apart	4 µg/mL erythromycin and 0.5 µg/mL clindamycin in same well	1 µg/mL erythromycin and 0.5 µg/mL clindamycin in same well
Inoculum	Standard disk diffusion procedure or heavily inoculated area of purity plate	Standard disk diffusion procedure	Standard broth microdilution procedure	
Incubation conditions	35°C ± 2°C; ambient air	35°C ± 2°C; 5% CO_2	35°C ± 2°C; ambient air	
Incubation length	16–18 hours	20–24 hours	18–24 hours	20–24 hours
Results	Flattening of the zone of inhibition adjacent to the erythromycin disk (referred to as a D-zone) = inducible clindamycin resistance. Hazy growth within the zone of inhibition around clindamycin = clindamycin resistance, even if no D-zone is apparent.		Any growth = inducible clindamycin resistance. No growth = no inducible clindamycin resistance.	

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Table 3G. (Continued)

Test	Inducible Clindamycin Resistance			
Test method	**Disk Diffusion (D-zone test)**		**Broth Microdilution**	
Organism group (applies only to organisms resistant to erythromycin and susceptible or intermediate to clindamycin)	*S. aureus, S. lugdunensis,* and CoNS	*S. pneumoniae* and β-hemolytic *Streptococcus* spp.	*S. aureus, S. lugdunensis,* and CoNS[b]	*S. pneumoniae* and β-hemolytic *Streptococcus* spp.
Additional testing and reporting	Report isolates with inducible clindamycin resistance as "clindamycin resistant." The following comment may be included with the report: "This isolate is presumed to be resistant based on detection of inducible clindamycin resistance."			
QC recommendations – routine[b]	*S. aureus* ATCC®[c] 25923 for routine QC of erythromycin and clindamycin disks	*S. pneumoniae* ATCC® 49619 for routine QC of erythromycin and clindamycin disks	*S. aureus* ATCC® BAA-976™[d] or *S. aureus* ATCC® 29213 – no growth	*S. pneumoniae* ATCC® 49619 or *S. aureus* ATCC® BAA-976™ – no growth
QC recommendations – lot/shipment[d]			*S. aureus* ATCC® BAA-977™ – growth	
QC recommendations – supplemental[e]	*S. aureus* ATCC® BAA-976™ (D-zone test negative) *S. aureus* ATCC® BAA-977™ (D-zone test positive) Use of unsupplemented MHA is acceptable for these strains.		*S. aureus* ATCC® BAA-976™ (no growth) *S. aureus* ATCC® BAA-977™ (growth)	

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; CoNS, coagulase-negative staphylococci; LHB, lysed horse blood; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; TSA, tryptic soy agar.

Footnotes

a. Antimicrobial susceptibility testing (AST) of β-hemolytic streptococci does not need to be performed routinely (see general comment [4] in Table 2H-1). When susceptibility testing is clinically indicated, it should include testing for inducible clindamycin resistance. In accordance with 2010 guidance from the Centers for Disease Control and Prevention, colonizing isolates of group B streptococci from penicillin-allergic pregnant women should be tested for inducible clindamycin resistance (see comment [12] in Table 2H-1).[1]

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Table 3G. (Continued)

b. QC recommendations – routine

Test negative (susceptible) QC strain:

- With each new lot/shipment of testing materials
- Weekly if the test is performed at least once a week and criteria for converting from daily to weekly QC testing have been met (see Subchapter 4.7.2.3 in M02[2] and M07[3])
- Daily if the test is performed less than once per week and/or if criteria for converting from daily to weekly QC testing have not been met

c. ATCC® is a registered trademark of the American Type Culture Collection. Per ATCC® convention, the trademark symbol is used after "BAA" in each catalog number, in conjunction with the registered ATCC® name.

d. QC recommendations – lot/shipment

Test positive (resistant) QC strain at minimum with each new lot/shipment of testing materials.

e. QC recommendations – supplemental

- Supplemental QC strains can be used to assess a new test, for training personnel, and for competence assessment. It is not necessary to include supplemental QC strains in routine daily or weekly AST QC programs. See Appendix C, which describes use of QC strains.

References for Table 3G

1 Verani JR, McGee L, Schrag SJ; Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention (CDC). Prevention of perinatal group B streptococcal disease – revised guidelines from CDC, 2010. *MMWR Recomm Rep.* 2010;59(RR-10):1-36.

2 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

3 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

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Table 3H. Test for Detection of High-Level Mupirocin Resistance in *Staphylococcus aureus*

Test	High-Level Mupirocin Resistance[a,1-3]	
Test method	**Disk diffusion**	**Broth microdilution**
Organism group	*S. aureus*	
Medium	MHA	CAMHB
Antimicrobial concentration	200-µg mupirocin disk	Single mupirocin 256-µg/mL well
Inoculum	Standard disk diffusion procedure	Standard broth microdilution procedure
Incubation conditions	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air
Incubation length	24 hours; read with transmitted light	24 hours
Results	Examine carefully with transmitted light for light growth within the zone of inhibition. No zone = high-level mupirocin resistance. Any zone = the absence of high-level mupirocin resistance.	For single 256-µg/mL well: Growth = high-level mupirocin resistance. No growth = the absence of high-level mupirocin resistance.
Additional testing and reporting	Report isolates with no zone as high-level mupirocin resistant. Report any zone of inhibition as the absence of high-level resistance.	Report growth in the 256-µg/mL well as high-level mupirocin resistant. Report no growth in the 256-µg/mL well as the absence of high-level resistance.
QC recommendations – routine[b]	*S. aureus* ATCC®[c] 25923 (200-µg disk) – *mupA* negative (zone 29–38 mm)	*S. aureus* ATCC® 29213 – *mupA* negative (MIC 0.06–0.5 µg/mL) or *E. faecalis* ATCC® 29212 – *mupA* negative (MIC 16–128 µg/mL)
QC recommendations – lot/shipment[d]	*S. aureus* ATCC® BAA-1708™ – *mupA* positive (no zone)	*S. aureus* ATCC® BAA-1708™ – *mupA* positive (growth in 256-µg/mL well)

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control.

Footnotes

a. Although not formally validated by CLSI document M23[1]–based analyses, some studies have linked a lack of response to mupirocin-based decolonization regimens with isolates for which the mupirocin MICs are ≥ 512 µg/mL.[2-4] Although this document does not provide guidance on breakpoints for mupirocin, disk-based testing and the MIC test described here identify isolates for which the mupirocin MICs are ≥ 512 µg/mL.

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Table 3H. (Continued)

b. QC recommendations – routine

Test negative (susceptible) QC strain:

- With each new lot/shipment of testing materials
- Weekly if the test is performed at least once a week and criteria for converting from daily to weekly QC testing have been met (see Subchapter 4.7.2.3 in M02[5] and M07[6])
- Daily if the test is performed less than once per week and/or if criteria for converting from daily to weekly QC testing have not been met

c. ATCC® is a registered trademark of the American Type Culture Collection. Per ATCC® convention, the trademark symbol is used after "BAA" in each catalog number, in conjunction with the registered ATCC® name.

d. QC recommendations – lot/shipment:

Test positive (resistant) QC strain at minimum with each new lot/shipment of testing materials.

References for Table 3H

1 CLSI. *Development of* In Vitro *Susceptibility Testing Criteria and Quality Control Parameters*. 5th ed. CLSI guideline M23. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

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5 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

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Table 3I. Test for Detection of High-Level Aminoglycoside Resistance in *Enterococcus* spp.[a] (Includes Disk Diffusion)

Test	Gentamicin HLAR			Streptomycin HLAR		
Test method	Disk diffusion	Broth microdilution	Agar dilution	Disk diffusion	Broth microdilution	Agar dilution
Medium	MHA	BHI[b] broth	BHI[b] agar	MHA	BHI[b] broth	BHI[b] agar
Antimicrobial concentration	120-µg gentamicin disk	Gentamicin, 500 µg/mL	Gentamicin, 500 µg/mL	300-µg streptomycin disk	Streptomycin, 1000 µg/mL	Streptomycin, 2000 µg/mL
Inoculum	Standard disk diffusion procedure	Standard broth dilution procedure	10 µL of a 0.5 McFarland suspension spotted onto agar surface	Standard disk diffusion procedure	Standard broth dilution procedure	10 µL of a 0.5 McFarland suspension spotted onto agar surface
Incubation conditions	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air
Incubation length	16–18 hours	24 hours	24 hours	16–18 hours	24–48 hours (if susceptible at 24 hours, reincubate)	24–48 hours (if susceptible at 24 hours, reincubate)
Results	6 mm = resistant 7–9 mm = inconclusive ≥ 10 mm = susceptible MIC correlates: R = > 500 µg/mL S = ≤ 500 µg/mL	Any growth = resistant	> 1 colony = resistant	6 mm = resistant 7–9 mm = inconclusive ≥ 10 mm = susceptible MIC correlates: R = > 1000 µg/mL (broth) and > 2000 µg/mL (agar) S = ≤ **1000** µg/mL (broth) and ≤ **2000** µg/mL (agar)	Any growth = resistant	> 1 colony = resistant
Additional testing and reporting	Resistant: is not synergistic with cell wall–active agent (eg, ampicillin, penicillin, and vancomycin). Susceptible: is synergistic with cell wall–active agent (eg, ampicillin, penicillin, and vancomycin) that is also susceptible. If disk diffusion result is inconclusive: perform an agar dilution or broth dilution MIC test to confirm. Strains of enterococci with ampicillin and penicillin MICs ≥ 16 µg/mL are categorized as resistant. However, enterococci with low levels of penicillin (MICs 16–64 µg/mL) or ampicillin (MICs 16–32 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, see Subchapter 3.12.2.3 in M07[1]) if high doses of penicillin or ampicillin are used. Enterococci possessing higher levels of penicillin (MICs ≥ 128 µg/mL) or ampicillin (MICs ≥ 64 µg/mL) resistance may not be susceptible to the synergistic effect.[2,3] Physicians' requests to determine the actual MIC of penicillin or ampicillin for blood and CSF isolates of enterococci should be considered.					
QC recommendations – routine[c]	*E. faecalis* ATCC®[d] 29212: 16–23 mm	*E. faecalis* ATCC® 29212 – Susceptible	*E. faecalis* ATCC® 29212 – Susceptible	*E. faecalis* ATCC® 29212: 14–20 mm	*E. faecalis* ATCC® 29212 – Susceptible	*E. faecalis* ATCC® 29212 – Susceptible
QC recommendations – lot/shipment[e]		*E. faecalis* ATCC® 51299 – Resistant	*E. faecalis* ATCC® 51299 – Resistant		*E. faecalis* ATCC® 51299 – Resistant	*E. faecalis* ATCC® 51299 – Resistant

Abbreviations: ATCC®, American Type Culture Collection; BHI, brain heart infusion; CSF, cerebrospinal fluid; HLAR, high-level aminoglycoside resistance; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control.

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Table 3l. (Continued)

Footnotes

a. Other aminoglycosides do not need to be tested, because their activities against enterococci are not superior to gentamicin and streptomycin.

b. BHI: even though not as widely available, dextrose phosphate agar and broth have been shown in limited testing to perform comparably.

c. QC recommendations – routine

Test negative (susceptible) QC strain:

- With each new lot/shipment of testing materials
- Weekly if the test is performed at least once a week and criteria for converting from daily to weekly QC testing have been met (see Subchapter 4.7.2.3 in M02[4] and M07[1])
- Daily if the test is performed less than once per week and/or if criteria for converting from daily to weekly QC testing have not been met

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

e. QC recommendations – lot/shipment

Test positive (resistant) QC strain at minimum with each new lot/shipment of testing materials.

NOTE: Information in boldface type is new or modified since the previous edition.

References for Table 3l

1 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

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4 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

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Table 4A-1. Disk Diffusion QC Ranges for Nonfastidious Organisms and Antimicrobial Agents Excluding β-Lactam Combination Agents[a]

Antimicrobial Agent	Disk Content	Disk Diffusion QC Ranges, mm		
		Escherichia coli ATCC®[b] 25922	*Staphylococcus aureus* ATCC® 25923	*Pseudomonas aeruginosa* ATCC® 27853
Amikacin	30 μg	19–26	20–26	18–26
Ampicillin	10 μg	15–22	27–35	–
Azithromycin	15 μg	–	21–26	–
Azlocillin	75 μg	–	–	24–30
Aztreonam	30 μg	28–36	–	23–29
Carbenicillin	100 μg	23–29	–	18–24
Cefaclor	30 μg	23–27	27–31	–
Cefamandole	30 μg	26–32	26–34	–
Cefazolin	30 μg	21–27	29–35	–
Cefdinir	5 μg	24–28	25–32	–
Cefditoren	5 μg	22–28	20–28	–
Cefepime	30 μg	31–37	23–29	25–31
Cefetamet	10 μg	24–29	–	–
Cefiderocol	**30 μg**	**25–31**	**–**	**22–31**
Cefixime	5 μg	**20–26**	–	–
Cefmetazole	30 μg	26–32	25–34	–
Cefonicid	30 μg	25–29	22–28	–
Cefoperazone	75 μg	28–34	24–33	23–29
Cefotaxime	30 μg	29–35	25–31	18–22
Cefotetan	30 μg	28–34	17–23	–
Cefoxitin	30 μg	23–29	23–29	–
Cefpodoxime	10 μg	23–28	19–25	–
Cefprozil	30 μg	21–27	27–33	–
Ceftaroline	30 μg	26–34	26–35	–
Ceftazidime	30 μg	25–32	16–20	22–29
Ceftibuten	30 μg	27–35	–	–
Ceftizoxime	30 μg	30–36	27–35	12–17
Ceftobiprole	30 μg	30–36	26–34	24–30
Ceftriaxone	30 μg	29–35	22–28	17–23
Cefuroxime	30 μg	20–26	27–35	–
Cephalothin	30 μg	15–21	29–37	–
Chloramphenicol	30 μg	21–27	19–26	–
Cinoxacin	100 μg	26–32	–	–
Ciprofloxacin	5 μg	**29–37**	22–30	25–33
Clarithromycin	15 μg	–	26–32	–
Clinafloxacin	5 μg	31–40	28–37	27–35
Clindamycin[c]	2 μg	–	24–30	–

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Table 4A-1. (Continued)

Antimicrobial Agent	Disk Content	Disk Diffusion QC Ranges, mm		
		Escherichia coli ATCC®[b] 25922	*Staphylococcus aureus* ATCC® 25923	*Pseudomonas aeruginosa* ATCC® 27853
Colistin	10 μg	11–17	–	11–17
Delafloxacin	5 μg	28–35[g]	32–40[g]	23–29[g]
Dirithromycin	15 μg	–	18–26	–
Doripenem	10 μg	27–35	33–42	28–35
Doxycycline	30 μg	18–24	23–29	–
Enoxacin	10 μg	28–36	22–28	22–28
Eravacycline	20 μg	16–23	19–26	–
Ertapenem	10 μg	29–36	24–31	13–21
Erythromycin[c]	15 μg	–	22–30	–
Faropenem	5 μg	20–26	27–34	–
Fleroxacin	5 μg	28–34	21–27	12–20
Fosfomycin[d]	200 μg	22–30	25–33	–
Fusidic acid	10 μg	–	24–32	–
Garenoxacin	5 μg	28–35	30–36	19–25
Gatifloxacin	5 μg	30–37	27–33	20–28
Gemifloxacin	5 μg	29–36	27–33	19–25
Gentamicin[e]	10 μg	19–26	19–27	17–23
Gepotidacin	10 μg	18–26	23–29	–
Grepafloxacin	5 μg	28–36	26–31	20–27
Iclaprim	5 μg	14–22	25–33	–
Imipenem	10 μg	26–32	–	20–28
Kanamycin	30 μg	17–25	19–26	–
Lefamulin	20 μg	–	26–32	–
Levofloxacin	5 μg	29–37	25–30	19–26
Levonadifloxacin	10 μg	27–33[g]	32–39[g]	17–23[g]
Linezolid	30 μg	–	25–32	–
Lomefloxacin	10 μg	27–33	23–29	22–28
Loracarbef	30 μg	23–29	23–31	–
Mecillinam	10 μg	24–30	–	–
Meropenem	10 μg	28–35	29–37	27–33
Methicillin	5 μg	–	17–22	–
Mezlocillin	75 μg	23–29	–	19–25
Minocycline	30 μg	19–25	25–30	–
Moxalactam	30 μg	28–35	18–24	17–25
Moxifloxacin	5 μg	28–35	28–35	17–25
Nafcillin	1 μg	–	16–22	–
Nafithromycin	15 μg	–	25–31[g]	–
Nalidixic acid	30 μg	22–28	–	–
Netilmicin	30 μg	22–30	22–31	17–23
Nitrofurantoin	300 μg	20–25	18–22	–

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Table 4A-1. (Continued)

Antimicrobial Agent	Disk Content	Disk Diffusion QC Ranges, mm		
		Escherichia coli **ATCC®[b] 25922**	***Staphylococcus aureus*** **ATCC® 25923**	***Pseudomonas aeruginosa*** **ATCC® 27853**
Norfloxacin	10 μg	28–35	17–28	22–29
Ofloxacin	5 μg	29–33	24–28	17–21
Omadacycline	30 μg	22–28	22–30	–
Oxacillin	1 μg	–	18–24	–
Pefloxacin	5 μg	25–33	–	–
Penicillin	10 units	–	26–37	–
Piperacillin	100 μg	24–30	–	25–33
Plazomicin	30 μg	21–27	19–25	15–21
Polymyxin B	300 units	13–19	–	14–18
Quinupristin-dalfopristin	15 μg	–	21–28	–
Razupenem	10 μg	21–26	–[f]	–
Rifampin	5 μg	8–10	26–34	–
Solithromycin	15 μg	–	22–30	–
Sparfloxacin	5 μg	30–38	27–33	21–29
Streptomycin[e]	10 μg	12–20	14–22	–
Sulfisoxazole[h]	250 μg or 300 μg	15–23	24–34	–
Tedizolid	20 μg	–	22–29	–
Teicoplanin	30 μg	–	15–21	–
Telithromycin	15 μg	–	24–30	–
Tetracycline	30 μg	18–25	24–30	–
Ticarcillin	75 μg	24–30	–	21–27
Tigecycline	15 μg	20–27	20–25	9–13
Tobramycin	10 μg	18–26	19–29	20–26
Trimethoprim[h]	5 μg	21–28	19–26	–
Trimethoprim-sulfamethoxazole[h]	1.25/23.75 μg	23–29	24–32	–
Trospectomycin	30 μg	10–16	15–20	–
Trovafloxacin	10 μg	29–36	29–35	21–27
Ulifloxacin (prulifloxacin)[i]	5 μg	32–38	20–26	27–33
Vancomycin	30 μg	–	17–21	–

Abbreviations: ATCC®, American Type Culture Collection, QC, quality control.

NOTE: Information in boldface type is new or modified since the previous edition.

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Table 4A-1. (Continued)

Footnotes

a. **Refer to Table 4A-2 for QC of β-lactam combination agents.**

b. ATCC® is a registered trademark of the American Type Culture Collection. Per ATCC® convention, the trademark symbol is used after "BAA" in each catalog number, in conjunction with the registered ATCC® name.

c. When disk approximation tests are performed with erythromycin and clindamycin, *S. aureus* ATCC® BAA-977™ (containing inducible *erm*(A)-mediated resistance) and *S. aureus* ATCC® BAA-976™ (containing *msr*(A)-mediated macrolide-only efflux) are recommended as supplemental QC strains (eg, for training, competence assessment, or test evaluation). *S. aureus* ATCC® BAA-977™ should demonstrate inducible clindamycin resistance (ie, a positive D-zone test), whereas *S. aureus* ATCC® BAA-976™ should not demonstrate inducible clindamycin resistance. *S. aureus* ATCC® 25923 should be used for routine QC (eg, weekly or daily) of erythromycin and clindamycin disks using standard Mueller-Hinton agar.

d. The 200-μg fosfomycin disk contains 50 μg of glucose-6-phosphate.

e. For control ranges of gentamicin 120-μg and streptomycin 300-μg disks, use *E. faecalis* ATCC® 29212 (gentamicin: 16–23 mm; streptomycin: 14–20 mm).

f. Razupenem tested with *S. aureus* ATCC® 25923 can often produce the double or target zone phenomenon. For accurate QC results, use *S. aureus* ATCC® 29213 (no double zones) with acceptable range 33–39 mm.

g. QC ranges for delafloxacin, levonadifloxacin, and nafithromycin were established using data from only one disk manufacturer. Disks from other manufacturers were not available at the time of testing.

h. These agents can be affected by excess levels of thymidine and thymine. See M02,[1] Subchapter 3.1.1.2 for guidance, should a problem with QC occur.

i. Ulifloxacin is the active metabolite of the prodrug prulifloxacin. Only ulifloxacin should be used for antimicrobial susceptibility testing.

Reference for Table 4A-1

1 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests.* 13th ed. CLSI standard M02. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

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Table 4A-2. Disk Diffusion QC Ranges for Nonfastidious Organisms and β-Lactam Combination Agents*

Antimicrobial Agent	Disk Content	QC Organisms and Characteristics							
		Escherichia coli ATCC®[a] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 25923	*Escherichia coli* ATCC®[b,c] 35218	*Klebsiella pneumoniae* ATCC® 700603[b,c]	*Escherichia coli* NCTC 13353[b,c]	*Klebsiella pneumoniae* ATCC® BAA-1705™[b,c]	*Klebsiella pneumoniae* ATCC® BAA-2814™
		β-lactamase negative	Inducible AmpC	β-lactamase negative, *mec*A negative	TEM-1	SHV-18 OXA-2 Mutations in OmpK35 and OmpK37 TEM-1	CTX-M-15	KPC-2 SHV	KPC-3 SHV-11 TEM-1
		MIC QC ranges, mm							
Amoxicillin-clavulanate (2:1)	20/10 μg	18–24	–	28–36	17–22	–	–	–	–
Ampicillin	10 μg	15–22	–	27–35	6	–	–	–	–
Ampicillin-sulbactam (2:1)	10/10 μg	19–24	–	29–37	13–19	–	–	–	–
Aztreonam	30 μg	28–36	23–29	–	31–38	10–16	–	–	–
Aztreonam-avibactam	30/20 μg	32–38	24–30	–	31–38	26–32[e]	–	–	–
Cefepime	30 μg	31–37	25–31	23–29	–	–	–	–	–
Cefepime-tazobactam[d]	30/20 μg	32–37	27–31	24–30	–	25–30[e]	27–31	–	–
Cefotaxime	30 μg	29–35	18–22	25–31	–	17–25	–	–	–
Cefpodoxime	10 μg	23–28	–	19–25	–	9–16	–	–	–
Ceftaroline	30 μg	26–34	–	26–35	–	–	–	–	–
Ceftaroline-avibactam	30/15 μg	27–34	17–26	25–34	27–35	21–27[e]	–	–	–
Ceftazidime	30 μg	25–32	22–29	16–20		10–18	–	–	–
Ceftazidime-avibactam	30/15 μg	27–35	25–31	16–22	28–35	21–27[e]	–	–	–
Ceftolozane-tazobactam	30/10 μg	24–32	25–31	10–18	25–31	17–25	–	–	–
Ceftriaxone	30 μg	29–35	17–23	22–28	–	16–24	–	–	–
Meropenem	10 μg	28–35	27–33	29–37	–	–	–	11–18[e]	6[e]
Meropenem-vaborbactam[d]	20/10 μg	31–37	29–35	32–38	–	29–35	–	21–27	16–20
Piperacillin	100 μg	24–30	25–33	–	12–18	–	–	–	–
Piperacillin-tazobactam	100/10 μg	24–30	25–33	27–36	24–30	–	–	–	–
Ticarcillin	75 μg	24–30	21–27	–	6	–	–	–	–
Ticarcillin-clavulanate	75/10 μg	24–30	20–28	29–37	21–25	–	–	–	–

*** Unsupplemented Mueller-Hinton medium. See Table 4A-1 for QC ranges for combination agents from other drug classes.**

Abbreviations: ATCC®, American Type Culture Collection; MIC, minimal inhibitory concentration; N/A, not applicable; NCTC, National Collection of Type Cultures; QC, quality control.

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Table 4A-2. (Continued)

QC strain selection codes: **QC strain is recommended for routine QC.**
Test one of these agents by a disk diffusion or MIC method to confirm the integrity of the respective QC strain.[b,c]

Footnotes

a. ATCC® is a registered trademark of the American Type Culture Collection. Per ATCC® convention, the trademark symbol is used after "BAA" in each catalog number, in conjunction with the registered ATCC® name.

b. Careful attention to organism maintenance (eg, minimal subcultures) and storage (eg, −60°C or below) is especially important for these QC strains because spontaneous loss of the plasmid encoding the β-lactamase has been documented. If stored at temperatures above −60°C or if repeatedly subcultured, these strains may lose their resistance characteristics and QC results may be outside the acceptable ranges.

c. To confirm the integrity of the QC strain, test one of the single β-lactam agents highlighted in orange by either a disk diffusion or MIC test method when the strain is first subcultured from a frozen or lyophilized stock culture. In some cases, only MIC ranges are available to accomplish this confirmation (see Table 5A-2). In-range results for the single agent indicate the QC strain is reliable for QC of β-lactam combination agents. It is not necessary to check the QC strain again with a single agent until a new frozen or lyophilized stock culture is put into use, providing recommendations for handling QC strains as described in M02[1] and M07[2] are followed.

d. Either strain highlighted in green may be used for routine QC of this antimicrobial agent.

e. QC ranges were established using data from only one disk manufacturer. Disks from other manufacturers were not available at the time of testing.

References for Table 4A-2

1 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests.* 13th ed. CLSI standard M02. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

2 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

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Table 4B. Disk Diffusion QC Ranges for Fastidious Organisms

Antimicrobial Agent	Disk Content	Disk Diffusion QC Ranges, mm			
		Haemophilus influenzae ATCC®[a] 49247	*Haemophilus influenzae* ATCC® 49766	*Neisseria gonorrhoeae* ATCC® 49226	*Streptococcus pneumoniae* ATCC® 49619[b]
Amoxicillin-clavulanate[c]	20/10 μg	15–23	–	–	–
Ampicillin	10 μg	13–21	–	–	30–36
Ampicillin-sulbactam	10/10 μg	14–22	–	–	–
Azithromycin	15 μg	13–21	–	–	19–25
Aztreonam	30 μg	30–38	–	–	–
Cefaclor	30 μg	–	25–31	–	24–32
Cefdinir	5 μg	–	24–31	40–49	26–31
Cefditoren	5 μg	25–34	–	–	27–35
Cefepime	30 μg	25–31	–	37–46	28–35
Cefetamet	10 μg	23–28	–	35–43	–
Cefixime	5 μg	25–33	–	37–45	16–23
Cefmetazole	30 μg	16–21	–	31–36	–
Cefonicid	30 μg	–	30–38	–	–
Cefotaxime	30 μg	31–39	–	38–48	31–39
Cefotetan	30 μg	–	–	30–36	–
Cefoxitin	30 μg	–	–	33–41	–
Cefpodoxime	10 μg	25–31	–	35–43	28–34
Cefprozil	30 μg	–	20–27	–	25–32
Ceftaroline	30 μg	29–39	–	–	31–41
Ceftaroline-avibactam[d]	30/15 μg	30–38	–	–	–
Ceftazidime	30 μg	27–35	–	35–43	–
Ceftazidime-avibactam[d]	30/20 μg	28–34	–	–	23–31
Ceftibuten	30 μg	29–36	–	–	–
Ceftizoxime	30 μg	29–39	–	42–51	28–34
Ceftobiprole[e]	30 μg	28–36	30–38	–	33–39
Ceftolozane-tazobactam[d]	30/10 μg	23–29	–	–	21–29
Ceftriaxone	30 μg	31–39	–	39–51	30–35
Cefuroxime	30 μg	–	28–36	33–41	–
Cephalothin	30 μg	–	–	–	26–32
Chloramphenicol	30 μg	31–40	–	–	23–27
Ciprofloxacin	5 μg	34–42	–	48–58	–
Clarithromycin	15 μg	11–17	–	–	25–31
Clinafloxacin	5 μg	34–43	–	–	27–34
Clindamycin	2 μg	–	–	–	19–25
Delafloxacin	5 μg	40–51	–	–	28–36[f]
Dirithromycin	15 μg	–	–	–	18–25
Doripenem	10 μg	21–31	–	–	30–38
Doxycycline	30 μg	–	–	–	25–34
Enoxacin	10 μg	–	–	43–51	–
Eravacycline	20 μg	–	–	–	23–30
Ertapenem[e]	10 μg	20–28	27–33	–	28–35
Erythromycin	15 μg	–	–	–	25–30

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Table 4B. (Continued)

Antimicrobial Agent	Disk Content	Disk Diffusion QC Ranges, mm			
		Haemophilus influenzae ATCC®[a] 49247	*Haemophilus influenzae* ATCC® 49766	*Neisseria gonorrhoeae* ATCC® 49226	*Streptococcus pneumoniae* ATCC® 49619[b]
Faropenem	5 μg	15–22	–	–	27–35
Fleroxacin	5 μg	30–38	–	43–51	–
Fusidic acid	10 μg	–	–	–	9–16
Garenoxacin	5 μg	33–41	–	–	26–33
Gatifloxacin	5 μg	33–41	–	45–56	24–31
Gemifloxacin	5 μg	30–37	–	–	28–34
Gepotidacin	10 μg	–	–	–	22–28
Grepafloxacin	5 μg	32–39	–	44–52	21–28
Iclaprim	5 μg	24–33	–	–	21–29
Imipenem	10 μg	21–29	–	–	–
Lefamulin	20 μg	22–28	–	–	19–27
Levofloxacin	5 μg	32–40	–	–	20–25
Levonadifloxacin	10 μg	33–41[f]	–	–	24–31[f]
Linezolid	30 μg	–	–	–	25–34
Lomefloxacin	10 μg	33–41	–	45–54	–
Loracarbef	30 μg	–	26–32	–	22–28
Meropenem	10 μg	20–28	–	–	28–35
Moxifloxacin	5 μg	31–39	–	–	25–31
Nafithromycin	15 μg	16–20[f]	–	–	25–31[f]
Nitrofurantoin	300 μg	–	–	–	23–29
Norfloxacin	10 μg	–	–	–	15–21
Ofloxacin	5 μg	31–40	–	43–51	16–21
Omadacycline	30 μg	21–29	–	–	24–32
Oxacillin	1 μg	–	–	–	≤ 12[g]
Penicillin	10 units	–	–	26–34	24–30
Piperacillin-tazobactam	100/10 μg	33–38	–	–	–
Quinupristin-dalfopristin	15 μg	15–21	–	–	19–24
Razupenem	10 μg	24–30	–	–	29–36
Rifampin	5 μg	22–30	–	–	25–30
Solithromycin	15 μg	16–23	–	33–43	25–33
Sparfloxacin	5 μg	32–40	–	43–51	21–27
Spectinomycin	100 μg	–	–	23–29	–
Tedizolid	20 μg	–	–	–	24–30
Telithromycin	15 μg	17–23	–	–	27–33
Tetracycline	30 μg	14–22	–	30–42	27–31
Tigecycline	15 μg	23–31	–	30–40	23–29
Trimethoprim-sulfamethoxazole	1.25/23.75 μg	24–32	–	–	20–28
Trospectomycin	30 μg	22–29	–	28–35	–
Trovafloxacin	10 μg	32–39	–	42–55	25–32
Vancomycin	30 μg	–	–	–	20–27

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Table 4B. (Continued)

Disk Diffusion Testing Conditions for Clinical Isolates and Performance of QC

Organism	*H. influenzae*	*N. gonorrhoeae*	Streptococci and *N. meningitidis*
Medium	HTM	GC agar base and 1% defined growth supplement. The use of a cysteine-free growth supplement is not required for disk diffusion testing.	MHA supplemented with 5% defibrinated sheep blood
Inoculum	Colony suspension	Colony suspension	Colony suspension
Incubation characteristics	5% CO_2; 16–18 hours; 35°C	5% CO_2; 20–24 hours; 35°C	5% CO_2; 20–24 hours; 35°C

Abbreviations: ATCC®, American Type Culture Collection; HTM, *Haemophilus* test medium; MHA, Mueller-Hinton agar; QC, quality control.

NOTE: Information in boldface type is new or modified since the previous edition.

Footnotes

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

b. Despite the lack of reliable disk diffusion breakpoints for *S. pneumoniae* with certain β-lactams, *S. pneumoniae* ATCC® 49619 is the strain designated for QC of all disk diffusion tests with all *Streptococcus* spp.

c. When testing *Haemophilus* on HTM incubated in ambient air, the acceptable QC limits for *E. coli* ATCC® 35218 are 17–22 mm for amoxicillin-clavulanate.

d. QC limits for *E. coli* ATCC® 35218 in HTM: ceftaroline-avibactam 26–34 mm; ceftazidime-avibactam 27–34 mm; ceftolozane-tazobactam 25–31 mm.

e. Either *H. influenzae* ATCC® 49247 or 49766 may be used for routine QC testing.

f. QC ranges for delafloxacin, levonadifloxacin, and nafithromycin were established using data from only one disk manufacturer. Disks from other manufacturers were not available at the time of testing.

g. Deterioration in oxacillin disk content is best assessed with QC organism *S. aureus* ATCC® 25923, with an acceptable zone diameter of 18–24 mm.

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Table 4C. Disk Diffusion: Reference Guide to QC Frequency

This table summarizes the suggested QC frequency when modifications are made to antimicrobial susceptibility test systems (refer to CLSI document EP23™[1]). It applies only to antimicrobial agents for which satisfactory results have been obtained with either the 15-replicate (3- × 5-day) plan or 20 or 30 consecutive test day plan. Otherwise QC is required each test day.

	Required QC Frequency			
Test Modification	**1 Day**	**5 Days**	**15-Replicate Plan or 20- or 30-Day Plan**	**Comments**
Disks				
Use new shipment or lot number.	X			
Use new manufacturer.	X			
Addition of new antimicrobial agent to existing system.			X	In addition, perform in-house verification studies.
Media (prepared agar plates)				
Use new shipment or lot number.	X			
Use new manufacturer.		X		
Inoculum preparation				
Convert inoculum preparation/ standardization to use of a device that has its own QC protocol.		X		**Example:** Convert from visual adjustment of turbidity to use of a photometric device for which a QC procedure is provided.
Convert inoculum preparation/ standardization to a method that depends on user technique.			X	**Example:** Convert from visual adjustment of turbidity to another method that is not based on a photometric device.
Measuring zones				
Change method of measuring zones.			X	**Example:** Convert from manual zone measurements to automated zone reader. In addition, perform in-house verification studies.
Instrument/software (eg, automated zone reader)				
Software update that affects AST results		X		Monitor all drugs, not just those implicated in software modification
Repair of instrument that affects AST results	X			Depending on extent of repair (eg, critical component such as the photographic device), additional testing may be appropriate (eg, 5 days).

Abbreviations: AST, antimicrobial susceptibility testing; QC, quality control.

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Table 4C. (Continued)

NOTE 1: QC can be performed before or concurrent with testing patient isolates. Patient results can be reported for that day if QC results are within the acceptable limits.

NOTE 2: Manufacturers of commercial or in-house-prepared tests should follow their own internal procedures and applicable regulations.

NOTE 3: For troubleshooting out-of-range results, refer to M02,[2] Subchapter 4.8 and M100 Table 4D. Additional information is available in Appendix C (eg, QC organism characteristics, QC testing recommendations).

NOTE 4: Broth, saline, and/or water used to prepare an inoculum does not need routine QC.

References for Table 4C

1 CLSI. *Laboratory Quality Control Based on Risk Management; Approved Guideline.* CLSI document EP23-A™. Wayne, PA: Clinical and Laboratory Standards Institute; 2011.

2 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

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Table 4D. Disk Diffusion: Troubleshooting Guide

This table provides guidance for troubleshooting and corrective action for out-of-range QC, primarily using antimicrobial susceptibility tests with MHA. Refer to M02,[1] Chapter 4, for additional information. Out-of-range QC tests **are often the result of contamination or the use of an incorrect QC strain; corrective action should first include repeating the test with a pure culture of a freshly subcultured QC strain**. If the issue is unresolved, this troubleshooting guide should be consulted regarding additional suggestions for troubleshooting out-of-range QC results **and unusual clinical isolate results**. In addition, **see general corrective action outlined in M02[1] and notify manufacturers of potential product problems.**

General Comment

(1) QC organism maintenance: Avoid repeated subcultures. Retrieve new QC strain from stock. If using lyophilized strains, follow the maintenance recommendations of the manufacturer. Store *E. coli* ATCC®* 35218 and *K. pneumoniae* ATCC® 700603 stock cultures at −60°C or below and prepare working cultures weekly (refer to M02,[1] Subchapter 4.4).

NOTE: Information in boldface type is new or modified since the previous edition.

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
β-LACTAMS				
Amoxicillin-clavulanate Ticarcillin-clavulanate	*E. coli* ATCC® 35218	Zone too small	Clavulanate is labile. Disk has lost potency.	Use alternative lot of disks. Check storage conditions and package integrity.
Ampicillin	*E. coli* ATCC® 35218	Zone too large (should be no zone—resistant)	Spontaneous loss of the plasmid encoding the β-lactamase	See general comment (1) on QC organism maintenance.
Aztreonam Cefotaxime Cefpodoxime Ceftazidime Ceftriaxone	*K. pneumoniae* ATCC® 700603	Zone too large	Spontaneous loss of the plasmid encoding the β-lactamase	See general comment (1) on QC organism maintenance.
Carbenicillin	*P. aeruginosa* ATCC® 27853	Zone too small	QC strain develops resistance after repeated subculture.	See general comment (1) on QC organism maintenance.
Cefotaxime-clavulanate Ceftazidime-clavulanate	*K. pneumoniae* ATCC® 700603	Negative ESBL test	Spontaneous loss of the plasmid encoding the β-lactamase	See general comment (1) on QC organism maintenance.
Penicillins	Any	Zone too large	pH of media too low	Acceptable pH range = 7.2–7.4 Avoid CO_2 incubation, which lowers pH.
Penicillins	Any	Zone too small	pH of media too high	Acceptable pH range = 7.2–7.4
β-lactam group	Any	Zone initially acceptable, but decreases to possibly be out of range over time	Imipenem, clavulanate, and cefaclor are especially labile. Disks have lost potency.	Use alternative lot of disks. Check storage conditions and package integrity.

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Table 4D. (Continued)

NON-β-LACTAMS				
Aminoglycosides Quinolones	Any	Zone too small	pH of media too low	Acceptable pH range = 7.2–7.4 Avoid CO_2 incubation, which lowers pH.
	Any	Zone too large	pH of media too high	Acceptable pH range = 7.2–7.4
Aminoglycosides	*P. aeruginosa* ATCC® 27853	Zone too small	Ca++ and/or Mg++ content too high	Use alternative lot of media.
Aminoglycosides	*P. aeruginosa* ATCC® 27853	Zone too large	Ca++ and/or Mg++ content too low	Use alternative lot of media.
Clindamycin Macrolides	*S. aureus* ATCC® 25923	Zone too small	pH of media too low	Acceptable pH range = 7.2–7.4 Avoid CO_2 incubation, which lowers pH.
	S. aureus ATCC® 25923	Zone too large	pH of media too high	Acceptable pH range = 7.2–7.4
Quinolones	Any	Zone too small	pH of media too low	Acceptable pH range = 7.2–7.4 Avoid CO_2 incubation, which lowers pH.
Quinolones	Any	Zone too large	pH of media too high	Acceptable pH range = 7.2–7.4
Tetracyclines	Any	Zone too large	pH of media too low	Acceptable pH range = 7.2–7.4 Avoid CO_2 incubation, which lowers pH.
Tetracyclines	Any	Zone too small	pH of media too high	Acceptable pH range = 7.2–7.4
Tetracyclines	Any	Zone too small	Ca++ and/or Mg++ content too high	Use alternative lot of media.
Tetracyclines	Any	Zone too large	Ca++ and/or Mg++ content too low	Use alternative lot of media.
Sulfonamides Trimethoprim Trimethoprim-sulfamethoxazole	*E. faecalis* ATCC® 29212	Zone ≤ 20 mm	Media too high in thymidine content	Use alternative lot of media.
ALL AGENTS				
Various	Various	Zone too small	Contamination Use of magnification to read zones	Measure zone edge with visible growth detected with unaided eye. Subculture to determine purity and repeat if necessary.
Various	Any	Inoculum too light Error in inoculum preparation Media depth too thin MHA nutritionally unacceptable	Repeat using McFarland 0.5 turbidity standard or standardizing device. Check expiration date and proper storage if using barium sulfate or latex standards. Use agar with depth approximately 4 mm. Recheck alternate lots of MHA.	
Various	Any	Many zones too small	Inoculum too heavy Error in inoculum preparation Media depth too thick	Repeat using McFarland 0.5 turbidity standard or standardizing device. Check expiration date and proper storage if using barium sulfate or latex standards. Use agar with depth approximately 4 mm. Recheck alternate lots of MHA.

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Table 4D. (Continued)

ALL AGENTS (Continued)				
Various	Any	One or more zones too small or too large	Measurement error Transcription error Random defective disk Disk not pressed firmly against agar	Recheck readings for measurement or transcription errors. Retest. If retest results are out of range and no errors are detected, initiate corrective action.
Various	Various	Zone too large	Did not include lighter growth in zone measurement (eg, double zone, fuzzy zone edge)	Measure zone edge with visible growth detected with unaided eye.
Various	*S. pneumoniae* ATCC® 49619	Zones too large Lawn of growth scanty	Inoculum source plate too old and contains too many nonviable cells. Plate used to prepare inoculum should be 18–20 hours.	Subculture QC strain and repeat QC test, or retrieve new QC strain from stock.
Various	Any	One QC strain is out of range, but other QC organism(s) is in range with the same antimicrobial agent.	One QC organism may be a better indicator of a QC problem.	Retest this strain to confirm reproducibility of acceptable results. Evaluate with alternative strains with known MICs. Initiate corrective action with problem QC strain/antimicrobial agents.
Various	Any	Two QC strains are out of range with the same antimicrobial agent.	A problem with the disk	Use alternative lot of disks. Check storage conditions and package integrity.
Various	Any	Zones overlap.	Too many disks per plate	Place no more than 12 disks on a 150-mm plate and 5 disks on a 100-mm plate; for some fastidious bacteria that produce large zones, use fewer.

Abbreviations: ATCC®, American Type Culture Collection; ESBL, extended-spectrum β-lactamase; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control.

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

Reference for Table 4D

1 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

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Table 5A-1. MIC QC Ranges for Nonfastidious Organisms and Antimicrobial Agents Excluding β-Lactam Combination Agents[a]

Antimicrobial Agent	MIC QC Ranges, µg/mL			
	Staphylococcus aureus ATCC®[b] 29213	*Enterococcus faecalis* ATCC® 29212	*Escherichia coli* ATCC® 25922	*Pseudomonas aeruginosa* ATCC® 27853
Amikacin	1–4	64–256	0.5–4	1–4
Amikacin-fosfomycin (5:2)[c]	0.5/0.2–4/1.6	32/12.8–128/51.2	0.25/0.1–2/0.8	1/0.4–8/3.2
Amoxicillin	–	–	–	–
Ampicillin	0.5–2	0.5–2	2–8	–
Azithromycin	0.5–2	–	–	–
Azlocillin	2–8	1–4	8–32	2–8
Aztreonam	–	–	0.06–0.25	2–8
Besifloxacin	0.016–0.06	0.06–0.25	0.06–0.25	1–4
Biapenem	0.03–0.12	–	0.03–0.12	0.5–2
Cadazolid	0.06–0.5	0.06–0.25	–	–
Carbenicillin	2–8	16–64	4–16	16–64
Cefaclor	1–4	–	1–4	–
Cefamandole	0.25–1	–	0.25–1	–
Cefazolin	0.25–1	–	1–4	–
Cefdinir	0.12–0.5	–	0.12–0.5	–
Cefditoren	0.25–2	–	0.12–1	–
Cefepime	1–4	–	0.016–0.12	0.5–4
Cefetamet	–	–	0.25–1	–
Cefiderocol[d]	**–**	**–**	**0.06–0.5**	**0.06–0.5**
Cefixime	8–32	–	0.25–1	–
Cefmetazole	0.5–2	–	0.25–1	>32
Cefonicid	1–4	–	0.25–1	–
Cefoperazone	1–4	–	0.12–0.5	2–8
Cefotaxime	1–4	–	0.03–0.12	8–32
Cefotetan	4–16	–	0.06–0.25	–
Cefoxitin	1–4	–	2–8	–
Cefpodoxime	1–8	–	0.25–1	–
Cefprozil	0.25–1	–	1–4	–
Ceftaroline	0.12–0.5	0.25–2[e]	0.03–0.12	–
Ceftazidime	4–16	–	0.06–0.5	1–4
Ceftibuten	–	–	0.12–0.5	–
Ceftizoxime	2–8	–	0.03–0.12	16–64
Ceftobiprole	0.12–1	0.06–0.5	0.03–0.12	1–4
Ceftriaxone	1–8	–	0.03–0.12	8–64
Cefuroxime	0.5–2	–	2–8	–
Cephalothin	0.12–0.5	–	4–16	–

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Table 5A-1. (Continued)

Antimicrobial Agent	MIC QC Ranges, µg/mL			
	Staphylococcus aureus ATCC®[b] 29213	*Enterococcus faecalis* ATCC® 29212	*Escherichia coli* ATCC® 25922	*Pseudomonas aeruginosa* ATCC® 27853
Chloramphenicol	2–16	4–16	2–8	–
Cinoxacin	–	–	2–8	–
Ciprofloxacin[f]	0.12–0.5	0.25–2	0.004–0.016	**0.12**–1
Clarithromycin	0.12–0.5	–	–	–
Clinafloxacin	0.008–0.06	0.03–0.25	0.002–0.016	0.06–0.5
Clindamycin[g]	0.06–0.25	4–16	–	–
Colistin	–	–	0.25–2	0.5–4
Dalbavancin[h]	0.03–0.12	0.03–0.12	–	–
Daptomycin[i]	0.12–1	1–4	–	–
Delafloxacin	0.001–0.008	0.016–0.12	0.008–0.03	0.12–0.5
Dirithromycin	1–4	–	–	–
Doripenem	0.016–0.06	1–4	0.016–0.06	0.12–0.5
Doxycycline	0.12–0.5	2–8	0.5–2	–
Enoxacin	0.5–2	2–16	0.06–0.25	2–8
Eravacycline	0.016–0.12	0.016–0.06	0.03–0.12	2–16
Ertapenem	0.06–0.25	4–16	0.004–0.016	2–8
Erythromycin[g]	0.25–1	1–4	–	–
Faropenem	0.03–0.12	–	0.25–1	–
Fidaxomicin	2–16	1–4	–	–
Finafloxacin	0.03–0.25	0.25–1	0.004–0.03	1–8
Fleroxacin	0.25–1	2–8	0.03–0.12	1–4
Fosfomycin[j]	0.5–4	32–128	0.5–2	2–8
Fusidic acid	0.06–0.25	–	–	–
Garenoxacin	0.004–0.03	0.03–0.25	0.004–0.03	0.5–2
Gatifloxacin	0.03–0.12	0.12–1.0	0.008–0.03	0.5–2
Gemifloxacin	0.008–0.03	0.016–0.12	0.004–0.016	0.25–1
Gentamicin[k]	0.12–1	4–16	0.25–1	0.5–2
Gepotidacin	0.12–1	–	1–4	–
Grepafloxacin	0.03–0.12	0.12–0.5	0.004–0.03	0.25–2.0
Iclaprim	0.06–0.25	0.004–0.03	1–4	–
Imipenem	0.016–0.06	0.5–2	0.06–0.25	1–4
Kanamycin	1–4	16–64	1–4	–
Lefamulin	0.06–0.25	–	–	–
Levofloxacin	0.06–0.5	0.25–2	0.008–0.06	0.5–4
Levonadifloxacin	0.008–0.03	–	0.03–0.25	0.5–4
Linezolid[l]	1–4	1–4	–	–
Lomefloxacin	0.25–2	2–8	0.03–0.12	1–4
Loracarbef	0.5–2	–	0.5–2	> 8

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Table 5A-1. (Continued)

Antimicrobial Agent	MIC QC Ranges, µg/mL			
	Staphylococcus aureus **ATCC®[b] 29213**	***Enterococcus faecalis*** **ATCC® 29212**	***Escherichia coli*** **ATCC® 25922**	***Pseudomonas aeruginosa*** **ATCC® 27853**
Mecillinam	–	–	0.03–0.25[m]	–
Meropenem	0.03–0.12	2–8	0.008–0.06	0.12–1
Methicillin	0.5–2	> 16	–	–
Mezlocillin	1–4	1–4	2–8	8–32
Minocycline[f]	0.06–0.5	1–4	0.25–1	–
Moxalactam	4–16	–	0.12–0.5	8–32
Moxifloxacin	0.016–0.12	0.06–0.5	0.008–0.06	1–8
Nafcillin	0.12–0.5	2–8	–	–
Nafithromycin	0.06–0.25	0.016–0.12	–	–
Nalidixic acid[f]	–	–	1–4	–
Netilmicin	≤ 0.25	4–16	≤ 0.5–1	0.5–8
Nitrofurantoin	8–32	4–16	4–16	–
Norfloxacin	0.5–2	2–8	0.03–0.12	1–4
Ofloxacin	0.12–1	1–4	0.016–0.12	1–8
Omadacycline[n]	0.12–1	0.06–0.5	0.25–2	–
Oritavancin[h]	0.016–0.12	0.008–0.03	–	–
Oxacillin	0.12–0.5	8–32	–	–
Penicillin	0.25–2	1–4	–	–
Pexiganan	8–32	16–64	2–8	2–16
Piperacillin	1–4	1–4	1–4	1–8
Plazomicin	0.25–2	32–128	0.25–2	1–4
Polymyxin B	–	–	0.25–2	0.5–2
Quinupristin-dalfopristin	0.25–1	2–8	–	–
Razupenem	0.008–0.03	0.25–1	0.06–0.5	–
Rifampin	0.004–0.016	0.5–4	4–16	16–64
Solithromycin	0.03–0.12	0.016–0.06	–	–
Sparfloxacin	0.03–0.12	0.12–0.5	0.004–0.016	0.5–2
Sulfisoxazole[f,o]	32–128	32–128	8–32	–
Sulopenem	0.016–0.12	2–8	0.016–0.06	–
Tedizolid[p]	0.12–1	0.25–1	–	–
Teicoplanin	0.25–1	0.25–1	–	–
Telavancin[h]	0.03–0.12	0.03–0.12	–	–
Telithromycin	0.06–0.25	0.016–0.12	–	–
Tetracycline	0.12–1	8–32	0.5–2	8–32
Ticarcillin	2–8	16–64	4–16	8–32
Tigecycline[n]	0.03–0.25	0.03–0.12	0.03–0.25	–
Tobramycin	0.12–1	8–32	0.25–1	0.25–1

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Table 5A-1. (Continued)

Antimicrobial Agent	MIC QC Ranges, µg/mL			
	***Staphylococcus aureus* ATCC®[b] 29213**	***Enterococcus faecalis* ATCC® 29212**	***Escherichia coli* ATCC® 25922**	***Pseudomonas aeruginosa* ATCC® 27853**
Trimethoprim[o]	1–4	0.12–0.5	0.5–2	> 64
Trimethoprim-sulfamethoxazole[o] (1:19)	≤ 0.5/9.5	≤ 0.5/9.5	≤ 0.5/9.5	8/152–32/608
Trospectomycin	2–16	2–8	8–32	–
Trovafloxacin	0.008–0.03	0.06–0.25	0.004–0.016	0.25–2
Ulifloxacin (prulifloxacin)[q]	–	–	0.004–0.016	0.12–0.5
Vancomycin[r]	0.5–2	1–4	–	–
Zidebactam	**–**	**–**	**0.06–0.25**	**1–8**

Abbreviations: ATCC®, American Type Culture Collection; MIC, minimal inhibitory concentration; QC, quality control.

NOTE 1: These MICs were obtained in several referral laboratories by dilution methods. If four or fewer concentrations are tested, QC may be more difficult.

NOTE 2: Information in boldface type is new or modified since the previous edition.

Footnotes

a. **Refer to Table 5A-2 for QC of β-lactam combination agents.**

b. ATCC® is a registered trademark of the American Type Culture Collection. Per ATCC® convention, the trademark symbol is used after "BAA" in each catalog number, in conjunction with the registered ATCC® name.

c. QC ranges reflect MICs obtained when medium is supplemented with 25 µg/mL of glucose-6-phosphate.

d. QC ranges reflect MICs obtained when cation-adjusted Mueller-Hinton broth (CAMHB) is iron depleted. Testing requires Mueller-Hinton broth (MHB) with iron at 0.03 mg/L or less, zinc at 0.5–1 mg/L, calcium at 20–25 mg/L, and magnesium 10–12.5 mg/L. The zinc, calcium, and magnesium are added back to the broth after cation depletion.

e. Testing this strain with this antimicrobial agent is considered supplemental QC only and is not required as routine user QC testing.

f. QC limits for *E. coli* ATCC® 25922 with ciprofloxacin, nalidixic acid, minocycline, and sulfisoxazole when tested in CAMHB with 2.5% to 5% lysed horse blood incubated either in ambient air or 5% CO_2 (when testing *N. meningitidis*) are the same as those listed in Table 5A-**1**.

g. When the erythromycin/clindamycin combination well for detecting inducible clindamycin resistance is used, *S. aureus* ATCC® BAA-977™ (containing inducible *erm*(A)-mediated resistance) and *S. aureus* ATCC® 29213 or *S. aureus* ATCC® BAA-976™ (containing *msr*(A)-mediated macrolide-only efflux) are recommended for QC purposes. *S. aureus* ATCC® BAA-977™ should demonstrate inducible clindamycin resistance (ie, growth in the well), whereas *S. aureus* ATCC® 29213 and *S. aureus* ATCC® BAA-976™ should not demonstrate inducible clindamycin resistance (ie, no growth in the well).

h. QC ranges reflect MICs obtained when CAMHB is supplemented with 0.002% polysorbate-80.

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Table 5A-1. (Continued)

i. QC ranges reflect MICs obtained when MHB is supplemented with calcium to a final concentration of 50 μg/mL. Agar dilution has not been validated for daptomycin.

j. The approved MIC susceptibility testing method is agar dilution. Agar media should be supplemented with 25 μg/mL of glucose-6-phosphate. Broth dilution should not be performed.

k. For control organisms for gentamicin and streptomycin high-level aminoglycoside tests for enterococci, see Table 3**I**.

l. QC range for *S. aureus* ATCC® 25923 with linezolid is 1–4 μg/mL; this strain exhibits less trailing, and MIC end points are easier to interpret. *S. aureus* ATCC® 25923 is considered a supplemental QC strain and is not required for routine QC of linezolid MIC tests.

m. This test should be performed by agar dilution only.

n. For broth microdilution testing of omadacycline and tigecycline, when MIC panels are prepared, the medium must be prepared fresh on the day of use. The medium must be no more than 12 hours old at the time the panels are made; however, the panels may then be frozen for later use.

o. Very medium-dependent, especially with enterococci.

p. QC range for *S. aureus* ATCC® 25923 with tedizolid is 0.12–0.5 μg/mL; this strain exhibits less trailing, and MIC end points are easier to interpret. *S. aureus* ATCC® 25923 is considered a supplemental QC strain and is not required for routine QC of tedizolid MIC tests.

q. Ulifloxacin is the active metabolite of the prodrug prulifloxacin. Only ulifloxacin should be used for antimicrobial susceptibility testing.

r. For QC organisms for vancomycin screen test for enterococci, see Table 3F.

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Table 5A-2. MIC QC Ranges for Nonfastidious Organisms and β-Lactam Combination Agents*

Antimicrobial Agent	QC Organisms and Characteristics									
	Escherichia coli ATCC®[a] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 29213	*Enterococcus faecalis* ATCC® 29212	*Escherichia coli* ATCC® 35218[b,c]	*Klebsiella pneumoniae* ATCC 700603[b,c]	*Escherichia coli* NCTC 13353[b,c]	*Klebsiella pneumoniae* ATCC® BAA-1705™[b,c]	*Klebsiella pneumoniae* ATCC® BAA-2814™	*A. baumannii* NCTC 13304[b,c]
	β-lactamase negative	Inducible Amp C	Weak β-lactamase *mecA* negative	–	TEM-1	SHV-18 OXA-2 Mutations in OmpK35 and OmpK37	CTX-M-15	KPC-2 TEM SHV	KPC-3 SHV-11 TEM-1	OXA-27
	MIC QC Ranges, µg/mL									
Amoxicillin	–	–	–	–	–	> 128	–	–	–	–
Amoxicillin-clavulanate (2:1)[d]	2/1–8/4	–	0.12/0.06–0.5/0.25	0.25/0.12–1.0/0.5	4/2–16/8	4/2–16/8	–	–	–	–
Ampicillin	2–8	–	0.5–2	0.5–2	> 32	> 128	–	–	–	–
Ampicillin-sulbactam (2:1)[d]	2/1–8/4	–	–	–	8/4–32/16	8/4–32/16	–	–	–	–
Aztreonam	0.06–0.25	2–8	–	–	0.03–0.12	8–64	–	–	–	–
Aztreonam-avibactam	0.03/4–0.12/4	2/4–8/4	–	–	0.016/4–0.06/4	0.06/4–0.5/4	–	–	–	–
Cefepime	0.016–0.12	0.5–4	1–4	–	–	0.5–2	64–256	–	–	16–128
Cefepime-tazobactam	0.03/8–0.12/8	0.5/8–4/8	1/8–4/8	–	–	**0.12/8–0.5/8**	0.06/8–0.25/8	–	–	–
Cefepime-zidebactam (1:1)	**0.016–0.06**	**0.5–2**	**–**	**–**	–	**0.06–0.25**	**0.06–0.5**	–	–	**4–16**
Zidebactam[e]	**0.06–0.25**	**1–8**	**–**	**–**	–	–	**0.06–0.5**	–	–	**≥128**
Cefotaxime	0.03–0.12	8–32	1–4	–	–	–	–	–	–	–
Cefpodoxime	0.25–1	–	1–8	–	–	–	–	–	–	–
Ceftaroline	0.03–0.12	–	0.12–0.5	0.25–2	–	2–8	–	–	–	–
Ceftaroline-avibactam	0.03/4–0.12/4	–	0.12/4–0.5/4	–	0.016/4–0.06/4	0.25/4–1/4	–	–	–	–
Ceftazidime	0.06–0.5	1–4	4–16	–	–	16–64	–	–	–	–
Ceftazidime-avibactam	0.06/4–0.5/4	0.5/4–4/4	4/4–16/4	–	0.03/4–0.12/4	0.25/4–2/4	–	–	–	–
Ceftolozane-tazobactam	0.12/4–0.5/4	0.25/4–1/4	16/4–64/4	–	0.06/4–0.25/4	0.5/4–2/4	–	–	–	–
Ceftriaxone	0.03–0.12	8–64	1–8	–	–	–	–	–	–	–
Imipenem	0.06–0.25	1–4	0.016–0.06	0.5–2	–	**0.03–0.25**	–	**4–16**	**16–64**	–
Imipenem-relebactam[d]	0.06/4–0.25/4	0.25/4–1/4	0.008/4–0.03/4	0.5/4–2/4	0.06/4–0.25/4	**0.03/4–0.25/4**	–	0.03/4–0.25/4	0.06/4–0.25/4	–
Meropenem	0.008–0.06	0.12–1	0.03–0.12	2–8	**0.008–0.06**	–	–	8–64	32–256	–
Meropenem-vaborbactam[d]	0.008/8–0.06/8	0.12/8–1/8	0.03/8–0.12/8	–	0.008/8–0.06/8	0.016/8–0.06/8	–	0.008/8–0.06/8	0.12/8–0.5/8	–

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Table 5A-2. (Continued)

Antimicrobial Agent	QC Organisms and Characteristics									
	Escherichia coli ATCC®[a] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 29213	*Enterococcus faecalis* ATCC® 29212	*Escherichia coli* ATCC® 35218[b,c]	*Klebsiella pneumoniae* ATCC 700603[b,c]	*Escherichia coli* NCTC 13353[b,c]	*Klebsiella pneumoniae* ATCC® BAA-1705™[b,c]	*Klebsiella pneumoniae* ATCC® BAA-2814™	*A. baumannii* NCTC 13304[b,c]
	β-lactamase negative	Inducible Amp C	Weak β-lactamase *mecA* negative	–	TEM-1	SHV-18 OXA-2 Mutations in OmpK35 and OmpK37	CTX-M-15	KPC-2 TEM SHV	KPC-3 SHV-11 TEM-1	OXA-27
	MIC QC Ranges, µg/mL									
Piperacillin	1–4	1–8	1–4	1–4	> 64	–	–	–	–	–
Piperacillin-tazobactam[d]	1/4–4/4	1/4–8/4	0.25/4–2/4	1/4–4/4	0.5/4–2/4	8/4–32/4	–	–	–	–
Ticarcillin	4–16	8–32	2–8	16–64	> 128	> 256	–	–	–	–
Ticarcillin-clavulanate[d]	4/2–16/2	8/2–32/2	0.5/2–2/2	16/2–64/2	8/2–32/2	32/2–128/2	–	–	–	–

[*] **Unsupplemented Mueller-Hinton medium (cation-adjusted if broth). See Table 5A-1 for QC ranges for combination agents from other drug classes.**
Abbreviations: ATCC®, American Type Culture Collection; MIC, minimal inhibitory concentration; NCTC, National Collection of Type Cultures; QC, quality control; R, resistant; S, susceptible.

QC strain selection codes: **QC strain is recommended for routine QC.**
Test one of these agents by a disk diffusion or MIC method to confirm the integrity of the respective QC strain.[b,c]

NOTE: Information in boldface type is new or modified since the previous edition.

Footnotes

a. ATCC® is a registered trademark of the American Type Culture Collection. Per ATCC® convention, the trademark symbol is used after "BAA" in each catalog number, in conjunction with the registered ATCC® name.

b. Careful attention to organism maintenance (eg, minimal subcultures) and storage (eg, −60°C or below) is especially important for these QC strains because spontaneous loss of the plasmid encoding the β-lactamase has been documented. If stored at temperatures above −60°C or if repeatedly subcultured, these strains may lose their resistance characteristics and QC results may be outside the acceptable ranges.

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Table 5A-2. (Continued)

c. To confirm the integrity of the QC strain, test one of the single β-lactam agents highlighted in orange by either a disk diffusion or MIC test method when the strain is first subcultured from a frozen or lyophilized stock culture. In-range results for the single agent indicate the QC strain is reliable for QC of β-lactam combination agents. It is not necessary to check the QC strain again with a single agent until a new frozen or lyophilized stock culture is put into use, providing recommendations for handling QC strains as described in M02[1] and M07[2] are followed. If the highest concentration tested on a panel is lower than the QC range listed for the particular antimicrobial agent and the MIC result obtained for the QC strain is interpreted as resistant, the QC strain can be considered reliable for QC of β-lactam combination agents (eg, ampicillin panel concentrations 1–16 µg/mL; ampicillin *Enterobacteriaceae* breakpoints [µg/mL]: ≤8 [S], 16 [I], ≥32 [R]; MIC of >16 µg/ml [R] would be acceptable for *K. pneumoniae* ATCC® 700603).

d. Either strain highlighted in green may be used for routine QC of this antimicrobial agent.

e. Not tested as a single agent routinely.

References for Table 5A-2

1 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

2 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

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Table 5B. MIC QC Ranges for Fastidious Organisms (Broth Dilution Methods)

Antimicrobial Agent	MIC QC Ranges, µg/mL		
	***Haemophilus influenzae* ATCC®[a] 49247**	***Haemophilus influenzae* ATCC® 49766**	***Streptococcus pneumoniae* ATCC® 49619**
Amikacin-fosfomycin (5:2)[b]	0.5/0.2–4/1.6	–	8/3.2–64/25.6
Amoxicillin[b]	–	–	0.03–0.12
Amoxicillin-clavulanate (2:1)[c]	2/1–16/8	–	0.03/0.016–0.12/0.06
Ampicillin	2–8	–	0.06–0.25
Ampicillin-sulbactam (2:1)	2/1–8/4	–	–
Azithromycin	1–4	–	0.06–0.25
Aztreonam	0.12–0.5	–	–
Besifloxacin	0.016–0.06	–	0.03–0.12
Cefaclor	–	1–4	1–4
Cefamandole	–	0.25–1	–
Cefdinir	–	0.12–0.5	0.03–0.25
Cefditoren	0.06–0.25	–	0.016–0.12
Cefepime	0.5–2	–	0.03–0.25
Cefepime-tazobactam	0.5/8–2/8	–	0.03/8–0.12/8
Cefetamet	0.5–2	–	0.5–2
Cefixime	0.12–1	–	–
Cefmetazole	2–16	–	–
Cefonicid	–	0.06–0.25	–
Cefotaxime	0.12–0.5	–	0.03–0.12
Cefotetan	–	–	–
Cefoxitin	–	–	–
Cefpirome	0.25–1	–	–
Cefpodoxime	0.25–1	–	0.03–0.12
Cefprozil	–	1–4	0.25–1
Ceftaroline	0.03–0.12	–	0.008–0.03
Ceftaroline-avibactam	0.016/4–0.12/4	–	–
Ceftazidime	0.12–1	–	–
Ceftazidime-avibactam[d]	0.06/4–0.5/4	0.016/4–0.06/4	0.25/4–2/4
Ceftibuten	0.25–1	–	–
Ceftizoxime	0.06–0.5	–	0.12–0.5
Ceftobiprole[e]	0.12–1	0.016–0.06	0.004–0.03
Ceftolozane-tazobactam	0.5/4–2/4	–	0.25/4–1/4
Ceftriaxone	0.06–0.25	–	0.03–0.12
Cefuroxime	–	0.25–1	0.25–1
Cephalothin	–	–	0.5–2
Chloramphenicol	0.25–1	–	2–8
Ciprofloxacin[f]	0.004–0.03	–	–
Clarithromycin	4–16	–	0.03–0.12
Clinafloxacin	0.001–0.008	–	0.03–0.12
Clindamycin	–	–	0.03–0.12
Dalbavancin[h]	–	–	0.008–0.03

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Table 5B. (Continued)

Antimicrobial Agent	MIC QC Ranges, µg/mL		
	***Haemophilus influenzae* ATCC®[a] 49247**	***Haemophilus influenzae* ATCC® 49766**	***Streptococcus pneumoniae* ATCC® 49619**
Daptomycin[i]	–	–	0.06–0.5
Delafloxacin	0.00025–0.001	–	0.004–0.016
Dirithromycin	8–32	–	0.06–0.25
Doripenem	–	0.06–0.25	0.03–0.12
Doxycycline	–	–	0.016–0.12
Enoxacin	–	–	–
Eravacycline	0.06–0.5	–	0.004–0.03
Ertapenem	–	0.016–0.06	0.03–0.25
Erythromycin	–	–	0.03–0.12
Faropenem	–	0.12–0.5	0.03–0.25
Finafloxacin	–	0.002–0.008	0.25–1
Fleroxacin	0.03–0.12	–	–
Fusidic acid	–	–	4–32
Garenoxacin	0.002–0.008	–	0.016–0.06
Gatifloxacin	0.004–0.03	–	0.12–0.5
Gemifloxacin	0.002–0.008	–	0.008–0.03
Gentamicin	–	–	–
Gepotidacin	0.25–1	–	0.06–0.25
Grepafloxacin	0.002–0.015	–	0.06–0.5
Iclaprim	0.12–1	–	0.03–0.12
Imipenem	–	0.25–1	0.03–0.12
Imipenem-relebactam	–	0.25/4–1/4	**0.016/4–0.12/4**
Lefamulin	0.5–2	–	0.06–0.5
Levofloxacin	0.008–0.03	–	0.5–2
Levonadifloxacin	0.008–0.06	–	0.12–0.5
Linezolid	–	–	0.25–2
Lomefloxacin	0.03–0.12	–	–
Loracarbef	–	0.5–2	2–8
Meropenem	–	0.03–0.12	0.03–0.25
Metronidazole	–	–	–
Minocycline[f]	–	–	–
Moxifloxacin	0.008–0.03	–	0.06–0.25
Nafithromycin	2–8	–	0.008–0.03
Nalidixic acid[f]	–	–	–
Nitrofurantoin	–	–	4–16
Norfloxacin	–	–	2–8
Ofloxacin	0.016–0.06	–	1–4
Omadacycline[g]	0.5–2	–	0.016–0.12

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Table 5B. (Continued)

Antimicrobial Agent	MIC QC Ranges, µg/mL		
	***Haemophilus influenzae* ATCC®[a] 49247**	***Haemophilus influenzae* ATCC® 49766**	***Streptococcus pneumoniae* ATCC® 49619**
Oritavancin[h]	–	–	0.001–0.004
Penicillin	–	–	0.25–1
Pexiganan	8–32	–	16–64
Piperacillin-tazobactam	0.06/4–0.5/4	–	–
Quinupristin-dalfopristin	2–8	–	0.25–1
Razupenem	–	0.008–0.03	0.008–0.06
Rifampin	0.25–1	–	0.016–0.06
Solithromycin	1–4	–	0.004–0.016
Sparfloxacin	0.004–0.016	–	0.12–0.5
Spectinomycin	–	–	–
Sulfisoxazole[f]	–	–	–
Sulopenem	–	0.06–0.25	0.03–0.12
Tedizolid	–	–	0.12–0.5
Telavancin[h]	–	–	0.004–0.016
Telithromycin	1–4	–	0.004–0.03
Tetracycline	4–32	–	0.06–0.5
Tigecycline[g]	0.06–0.5	–	0.016–0.12
Trimethoprim-sulfamethoxazole (1:19)	0.03/0.59–0.25/4.75	–	0.12/2.4–1/19
Trospectomycin	0.5–2	–	1–4
Trovafloxacin	0.004–0.016	–	0.06–0.25
Vancomycin	–	–	0.12–0.5

NOTE: Information in boldface type is new or modified since the previous edition.

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Table 5B. (Continued)

Testing Conditions for Clinical Isolates and Performance of QC

Organism	*Haemophilus influenzae*	*Streptococcus pneumoniae* and streptococci	*Neisseria meningitidis*
Medium	Broth dilution: HTM broth	Broth dilution: CAMHB with LHB (2.5% to 5% v/v)	Broth dilution: CAMHB with LHB (2.5% to 5% v/v)
Inoculum	Colony suspension	Colony suspension	Colony suspension
Incubation characteristics	Ambient air; 20–24 hours; 35°C	Ambient air; 20–24 hours; 35°C	5% CO_2; 20–24 hours; 35°C (for QC with *S. pneumoniae* ATCC® 49619, 5% CO_2 or ambient air, except for azithromycin, ambient air only)

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; HTM, *Haemophilus* test medium; LHB, lysed horse blood; MIC, minimal inhibitory concentration; QC, quality control.

NOTE 1: Information in boldface type is new or modified since the previous edition.

NOTE 2: For four-dilution ranges, results at the extremes of the acceptable ranges should be suspect. Verify validity with data from other QC strains.

Footnotes

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

b. QC ranges reflect MICs obtained when medium is supplemented with 25 μg/mL of glucose-6-phosphate.

c. QC limits for *E. coli* ATCC® 35218 when tested on HTM are 4/2–16/8 μg/mL for amoxicillin-clavulanate and ≥ 256 μg/mL for amoxicillin; testing amoxicillin may help to determine if the isolate has maintained its ability to produce β-lactamase.

d. QC limits for *K. pneumoniae* ATCC® 700603 with ceftazidime-avibactam when testing in HTM are 0.25/4–1/4 μg/mL. *K. pneumoniae* ATCC® 700603 should be tested against ceftazidime-avibactam and ceftazidime alone to confirm the activity of avibactam in the combination and to ensure that the plasmid encoding the β-lactamase has not been lost in this strain. The acceptable range for ceftazidime alone is > 16 μg/mL.

e. Either *H. influenzae* ATCC® 49247 or 49766 may be used for routine QC testing.

f. QC limits for *E. coli* ATCC® 25922 with ciprofloxacin, nalidixic acid, minocycline, and sulfisoxazole when tested in CAMHB with 2.5% to 5% LHB incubated either in ambient air or 5% CO_2 (when testing *N. meningitidis*) are the same as those listed in Table 5A-**1**.

g. For broth microdilution testing of omadacycline and tigecycline, when MIC panels are prepared, the medium must be prepared fresh on the day of use. The medium must be no more than 12 hours old at the time the panels are made; however, the panels may then be frozen for later use.

h. QC ranges reflect MICs obtained when CAMHB is supplemented with 0.002% polysorbate-80.

i. QC ranges reflect MICs obtained when Mueller-Hinton broth is supplemented with calcium to a final concentration of 50 μg/mL. Agar dilution has not been validated for daptomycin.

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Table 5C. MIC QC Ranges for *Neisseria gonorrhoeae* (Agar Dilution Method)

Antimicrobial Agent	MIC QC Ranges, µg/mL *Neisseria gonorrhoeae* ATCC®[a] 49226
Azithromycin	0.25–1
Cefdinir	0.008–0.03
Cefepime	0.016–0.06
Cefetamet	0.016–0.25
Cefixime	0.004–0.03
Cefmetazole	0.5–2
Cefotaxime	0.016–0.06
Cefotetan	0.5–2
Cefoxitin	0.5–2
Cefpodoxime	0.03–0.12
Ceftazidime	0.03–0.12
Ceftizoxime	0.008–0.03
Ceftriaxone	0.004–0.016
Cefuroxime	0.25–1
Ciprofloxacin	0.001–0.008
Enoxacin	0.016–0.06
Fleroxacin	0.008–0.03
Gatifloxacin	0.002–0.016
Gepotidacin	0.25–1
Grepafloxacin	0.004–0.03
Lomefloxacin	0.008–0.03
Moxifloxacin	0.008–0.03
Ofloxacin	0.004–0.016
Penicillin	0.25–1
Solithromycin	0.03–0.25
Sparfloxacin	0.004–0.016
Spectinomycin	8–32
Tetracycline	0.25–1
Trospectomycin	1–4
Trovafloxacin	0.004–0.016

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Table 5C. (Continued)

Testing Conditions for Clinical Isolates and Performance of QC

Organism	*Neisseria gonorrhoeae*
Medium	Agar dilution: GC agar base and 1% defined growth supplement. The use of a cysteine-free supplement is necessary for agar dilution tests with carbapenems and clavulanate. Cysteine-containing defined growth supplements do not significantly alter dilution test results with other drugs.
Inoculum	Colony suspension, equivalent to a 0.5 McFarland standard
Incubation characteristics	36°C ± 1°C (do not exceed 37°C); 5% CO_2; 20–24 hours

Abbreviations: ATCC®, American Type Culture Collection; MIC, minimal inhibitory concentration; QC; quality control.

NOTE: For four-dilution ranges, results at the extremes of the acceptable ranges should be suspect. Verify validity with data from other QC strains.

Footnote

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

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Table 5D. MIC QC Ranges for Anaerobes (Agar Dilution Method)

Antimicrobial Agent	MIC QC Ranges, µg/mL			
	Bacteroides fragilis **ATCC®[a] 25285**	***Bacteroides thetaiotaomicron*** **ATCC® 29741**	***Clostridioides* (formerly *Clostridium*) *difficile*** **ATCC® 700057**	***Eggerthella lenta* (formerly *Eubacterium lentum*)** **ATCC® 43055[b]**
Amoxicillin-clavulanate (2:1)	0.25/0.125–1/0.5	0.5/0.25–2/1	0.25/0.125–1/0.5	–
Ampicillin	16–64	16–64	1–4	–
Ampicillin-sulbactam (2:1)	0.5/0.25–2/1	0.5/0.25–2/1	0.5/0.25–4/2	0.25/0.125–2/1
Cadazolid	–	–	0.12–0.5	–
Cefmetazole	8–32	32–128	–	4–16
Cefoperazone	32–128	32–128	–	32–128
Cefotaxime	8–32	16–64	–	64–256
Cefotetan	4–16	32–128	–	32–128
Cefoxitin	4–16	8–32	–	4–16
Ceftaroline	4–32	16–128	2–16	8–32
Ceftaroline-avibactam	0.12/4–0.5/4	4/4–16/4	0.5/4–4/4	4/4–16/4
Ceftizoxime	–	4–16	–	16–64
Ceftolozane-tazobactam	0.12/4–1/4	16/4–128/4	–	–
Ceftriaxone	32–128	64–256	–	–
Chloramphenicol	2–8	4–16	–	–
Clinafloxacin	0.03–0.125	0.06–0.5	–	0.03–0.125
Clindamycin	0.5–2	2–8	2–8	0.06–0.25
Doripenem	–	–	0.5–4	–
Eravacycline	0.06–0.25	0.12–1	0.06–0.25	–
Ertapenem	0.06–0.25	0.25–1	–	0.5–2
Faropenem	0.03–0.25	0.12–1	–	1–4
Fidaxomicin	–	–	0.06–0.25	–
Finafloxacin	0.12–0.5	1–4	1–4	0.12–0.5
Garenoxacin	0.06–0.5	0.25–1	0.5–2	1–4
Imipenem	0.03–0.125	0.125–0.5	–	0.125–0.5
Imipenem-relebactam	**0.03/4–0.25/4**	**0.06/4–0.5/4**	**–**	**0.12/4–1/4**
Linezolid	2–8	2–8	1–4	0.5–2
Meropenem	0.03–0.25	0.125–0.5	0.5–4	0.125–1
Metronidazole	0.25–1	0.5–2	0.125–0.5	–
Mezlocillin	16–64	8–32	–	8–32
Moxifloxacin	0.125–0.5	1–4	1–4	0.125–0.5
Nitazoxanide	–	–	0.06–0.5	–
Omadacycline	0.25–2	0.5–4	0.25–2	0.25–2
Penicillin	8–32	8–32	1–4	–
Piperacillin	2–8	8–32	4–16	8–32
Piperacillin-tazobactam	0.125/4–0.5/4	4/4–16/4	4/4–16/4	4/4–16/4

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Table 5D. (Continued)

Antimicrobial Agent	MIC QC Ranges, μg/mL			
	Bacteroides fragilis **ATCC®[a] 25285**	***Bacteroides thetaiotaomicron*** **ATCC® 29741**	***Clostridioides* (formerly *Clostridium*) *difficile*** **ATCC® 700057**	***Eggerthella lenta* (formerly *Eubacterium lentum*)** **ATCC® 43055[b]**
Ramoplanin	–	–	0.125–0.5	–
Razupenem	0.016–0.12	0.06–0.25	0.06–0.25	0.06–0.5
Ridinilazole	**–**	**–**	**0.06–0.25**	**–**
Rifaximin	–	–	0.004–0.016	–
Secnidazole	0.25–1	0.5–2	0.06–0.5	0.25–2
Sulopenem	–	0.06–0.5	1–4	0.5–2
Surotomycin[c]	–	–	0.12–1	2–8
Tetracycline	0.125–0.5	8–32	–	–
Ticarcillin	16–64	16–64	16–64	16–64
Ticarcillin-clavulanate	–	0.5/2–2/2	16/2–64/2	16/2–64/2
Tigecycline	0.12–1	0.5–2	0.125–1	0.06–0.5
Tinidazole	–	–	0.125–0.5	–
Tizoxanide	–	–	0.06–0.5	–
Vancomycin	–	–	0.5–4	–

Abbreviations: ATCC®, American Type Culture Collection; MIC, minimal inhibitory concentration; QC, quality control.

NOTE: Information in boldface type is new or modified since the previous edition.

Footnotes

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

b. MIC variability with some agents has been reported with *Eggerthella lenta* (formerly *E. lentum*) ATCC® 43055; therefore, QC ranges have not been established for all antimicrobial agents with this organism.

c. QC ranges reflect MICs obtained when media are supplemented with calcium to a final concentration of 50 μg/mL.

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Table 5E. MIC QC Ranges for Anaerobes (Broth Microdilution Method)

Antimicrobial Agent	MIC QC Ranges, µg/mL			
	Bacteroides fragilis **ATCC®[a] 25285**	***Bacteroides thetaiotaomicron*** **ATCC® 29741**	***Clostridioides* (formerly *Clostridium*) *difficile*** **ATCC® 700057**	***Eggerthella lenta* (formerly *Eubacterium lentum*)** **ATCC® 43055[b]**
Amoxicillin-clavulanate (2:1)	0.25/0.125–1/0.5	0.25/0.125–1/0.5	–	–
Ampicillin-sulbactam (2:1)	0.5/0.25–2/1	0.5/0.25–2/1	–	0.5/0.25–2/1
Cadazolid	–	–	0.06–0.25	–
Cefotetan	1–8	16–128	–	16–64
Cefoxitin	2–8	8–64	–	2–16
Ceftaroline	2–16	8–64	0.5–4	–
Ceftaroline-avibactam	0.06/4–0.5/4	2/4–8/4	0.25/4–1/4	4/4–16/4
Ceftizoxime	–	–	–	8–32
Ceftolozane-tazobactam	0.12/4–1/4	16/4–64/4	–	–
Chloramphenicol	4–16	8–32	–	4–16
Clindamycin	0.5–2	2–8	–	0.06–0.25
Doripenem	0.12–0.5	0.12–1	–	–
Doxycycline	–	2–8	–	2–16
Eravacycline	0.016–0.12	0.06–0.25	0.016–0.06	–
Ertapenem	0.06–0.5	0.5–2	–	0.5–4
Faropenem	0.016–0.06	0.12–1	–	0.5–2
Garenoxacin	0.06–0.25	0.25–2	–	0.5–2
Imipenem	0.03–0.25	0.25–1	–	0.25–2
Imipenem-relebactam	**0.03/4–0.125/4**	**–**	**–**	**–**
Linezolid	2–8	2–8	–	0.5–2
Meropenem	0.03–0.25	0.06–0.5	–	0.125–1
Metronidazole	0.25–2	0.5–4	–	0.125–0.5
Moxifloxacin	0.12–0.5	1.0–8	–	0.12–0.5
Omadacycline[c]	0.12–1	0.25–1	0.06–0.25	0.06–5
Penicillin	8–32	8–32	–	–
Piperacillin	4–16	8–64	–	8–32
Piperacillin-tazobactam	0.03/4–0.25/4	2/4–16/4	–	8/4–32/4
Razupenem	0.03–0.25	0.12–0.5	0.06–0.5	0.12–0.5
Ridinilazole	**–**	**–**	**0.12–0.5**	**–**
Sulopenem	–	0.03–0.25	0.5–2	0.25–1
Surotomycin[d]	–	–	0.12–1	1–4
Ticarcillin-clavulanate	0.06/2–0.5/2	0.5/2–2/2	–	8/2–32/2
Tigecycline[c]	0.06–0.5	0.25–1	0.03–0.12	–

Abbreviations: ATCC®, American Type Culture Collection; MIC, minimal inhibitory concentration; QC, quality control.

NOTE 1: Information in boldface type is new or modified since the previous edition.

NOTE 2: For four-dilution ranges, results at the extremes of the acceptable range(s) should be suspect. Verify validity with data from other QC strains.

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Table 5E. (Continued)

Footnotes

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

b. MIC variability with some agents has been reported with *Eggerthella lenta* (formerly *E. lentum*) ATCC® 43055; therefore, QC ranges have not been established for all antimicrobial agents with this organism.

c. For broth microdilution testing of omadacycline and tigecycline, when MIC panels are prepared, the medium must be prepared fresh on the day of use. The medium must be no greater than 12 hours old at the time the panels are made; however, the panels may then be frozen for later use.

d. QC ranges reflect MICs obtained when broth is supplemented with calcium to a final concentration of 50 μg/mL.

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Table 5F. MIC Reference Guide to QC Frequency

This table summarizes the suggested QC frequency when modifications are made to antimicrobial susceptibility test systems (refer to CLSI document EP23[1]). It applies only to antimicrobial agents for which satisfactory results have been obtained with either the 15-replicate (3- × 5-day) plan or 20 or 30 consecutive test day plan. Otherwise QC is required each test day.

	Required QC Frequency			
Test Modification	**1 Day**	**5 Days**	**15-Replicate Plan or 20- or 30-Day Plan**	**Comments**
MIC test(s)				
Use new shipment or lot number.	X			
Expand dilution range.	X			**Example:** Convert from breakpoint to expanded range MIC panels.
Reduce dilution range.	X			**Example:** Convert from expanded dilution range to breakpoint panels.
Use new method (same company).			X	**Examples:** Convert from overnight to rapid MIC test. In addition, perform in-house verification studies.
Use new manufacturer of MIC test.			X	In addition, perform in-house verification studies.
Use new manufacturer of broth or agar.		X		
Addition of new antimicrobial agent to existing system			X	In addition, perform in-house verification studies.
Inoculum preparation				
Convert inoculum preparation/standardization to use of a device that has its own QC protocol.		X		**Example**: Convert from visual adjustment of turbidity to use of a photometric device for which a QC procedure is provided.
Convert inoculum preparation/standardization to a method that depends on user technique.			X	**Example:** Convert from visual adjustment of turbidity to another method that is not based on a photometric device.
Instrument/software				
Software update that affects AST results		X		Monitor all drugs, not just those implicated in software modification.
Repair of instrument that affects AST results	X			Depending on extent of repair (eg, critical component such as the photographic device), additional testing may be appropriate (eg, 5 days).

Abbreviations: AST, antimicrobial susceptibility testing; MIC, minimal inhibitory concentration; QC, quality control.

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Table 5F. (Continued)

NOTE 1: QC can be performed before or concurrent with testing patient isolates. Patient results can be reported for that day if QC results are within the acceptable limits.

NOTE 2: Manufacturers of commercial or in-house-prepared tests should follow their own internal procedures and applicable regulations.

NOTE 3: Acceptable MIC QC limits for US Food and Drug Administration–cleared antimicrobial susceptibility tests may differ slightly from acceptable CLSI QC limits. Users of each device should use the manufacturer's procedures and QC limits as indicated in the instructions for use.

NOTE 4: For troubleshooting out-of-range results, refer to M07,[2] Subchapter 4.8 and M100 Table 5G. Additional information is available in Appendix C (eg, organism characteristics, QC testing recommendations).

NOTE 5: Broth, saline, and/or water used to prepare an inoculum does not need routine QC.

References for Table 5F

1 CLSI. *Laboratory Quality Control Based on Risk Management; Approved Guideline*. CLSI document EP23-A™. Wayne, PA: Clinical and Laboratory Standards Institute; 2011.

2 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

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Table 5G. MIC: Troubleshooting Guide

This table provides guidance for troubleshooting and corrective action for out-of-range QC, primarily using CAMHB for broth microdilution. Refer to M07,[1] Chapter 4, **for additional information**. Out-of-range QC tests are often the result of contamination or the use of an incorrect QC strain; corrective action should first include repeating the test with a pure culture of a freshly subcultured QC strain. If the issue is unresolved, this troubleshooting guide should be consulted regarding additional suggestions for troubleshooting out-of-range QC results and unusual clinical isolate results. In addition, see general corrective action outlined in M07[1] and notify manufacturers of potential product problems.

General Comment

(1) QC organism maintenance: Avoid repeated subcultures. Retrieve new QC strain from stock. If using lyophilized strains, follow the maintenance recommendations of the manufacturer. Store *E. coli* ATCC®* 35218 and *K. pneumoniae* ATCC® 700603 stock cultures at −60°C or below and prepare working cultures weekly (refer to M07,[1] Subchapter 4.4).

NOTE: Information in boldface type is new or modified since the previous edition.

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
β-LACTAMS				
Amoxicillin-clavulanate Ticarcillin-clavulanate	*E. coli* ATCC® 35218 *K. pneumoniae* ATCC® 700603	MIC too high	Clavulanate is labile. Antimicrobial agent is degrading.	Use alternative lot. Check storage conditions and package integrity.
Aztreonam Cefotaxime Cefpodoxime Ceftazidime Ceftriaxone	*K. pneumoniae* ATCC® 700603	MIC too low	Spontaneous loss of the plasmid encoding the β-lactamase	See general comment (1) on QC organism maintenance.
Carbenicillin	*P. aeruginosa* ATCC® 27853	MIC too high	QC strain develops resistance after repeated subculture.	See general comment (1) on QC organism maintenance.
Cefotaxime- clavulanate Ceftazidime-clavulanate	*K. pneumoniae* ATCC® 700603	Negative ESBL test	Spontaneous loss of the plasmid encoding the β-lactamase	See general comment (1) on QC organism maintenance.
Carbapenems	*P. aeruginosa* ATCC® 27853	MIC too high	Zn++ concentration in media is too high.	Use alternative lot.
Carbapenems	*P. aeruginosa* ATCC® 27853	MIC too high	Antimicrobial agent is degrading.	Use alternative lot. Check storage conditions and package integrity. Repeated imipenem QC results at the upper end of QC range with *P. aeruginosa* ATCC® 27853 may indicate deterioration of the drug.
Penicillin	*S. aureus* ATCC® 29213	MIC too high	QC strain is a β-lactamase producer; overinoculation may yield increased MICs.	Repeat with a carefully adjusted inoculum.
Penicillins	Any	MIC too low	pH of media too low	Acceptable pH range = 7.2–7.4 Avoid CO_2 incubation, which lowers pH.

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Table 5G. (Continued)

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
β-LACTAMS (Continued)				
Penicillins	Any	MIC too high	pH of media too high	Acceptable pH range = 7.2–7.4
β-Lactam group	Any	MIC initially acceptable, but increases to possibly be out of range over time	Imipenem, cefaclor, and clavulanate are especially labile. Antimicrobial agents are degrading.	Use alternative lot. Check storage and package integrity.
NON-β-LACTAMS				
Aminoglycosides Quinolones	Any	MIC too high	pH of media too low	Acceptable pH range = 7.2–7.4 Avoid CO_2 incubation, which lowers pH.
Aminoglycosides Quinolones	Any	MIC too low	pH of media too high	Acceptable pH range = 7.2–7.4
Aminoglycosides	*P. aeruginosa* ATCC® 27853	MIC too low	Ca++ and/or Mg++ content too low	Acceptable range = Ca++ 20–25 mg/L Mg++ 10–12.5 mg/L
Aminoglycosides	*P. aeruginosa* ATCC® 27853	MIC too low	Ca++ and/or Mg++ content too low	Acceptable range = Ca++ 20–25 mg/L Mg++ 10–12.5 mg/L
Dalbavancin Oritavancin[1] Telavancin	*S. aureus* ATCC® 29213 *E. faecalis* ATCC® 29212	MIC too high	Lack of polysorbate-80 in the media	Add polysorbate-80 to CAMHB to final concentration of 0.002% (v/v). See M07,[1] Subchapter 3.5.1 and Appendix A.
Chloramphenicol Clindamycin Erythromycin Linezolid Tedizolid Tetracycline	*S. aureus* ATCC® 29213 *E. faecalis* ATCC® 29212 *S. pneumoniae* ATCC® 49619	MIC too high	Trailing end point	Read at first well where the trailing begins; tiny buttons of growth should be ignored. See general comment (2) in Table 2G.
Linezolid Tedizolid	*S. aureus* ATCC® 29213	MIC too high	Trailing end point	*S. aureus* ATCC®25923 may be used as a supplemental QC strain for these drugs. This strain exhibits less trailing and MIC end points are easier to interpret.
Oritavancin[1]	*S. aureus* ATCC® 29213 *E. faecalis* ATCC® 29212	MIC too high	Lack of polysorbate-80 in the solvent and diluent	Dissolve antimicrobial powder and prepare dilutions in water containing a final concentration of 0.002% polysorbate-80 (v/v).
Oritavancin	*S. aureus* ATCC® 29213 *E. faecalis* ATCC® 29212	MIC too high	Use of tissue-culture treated microdilution trays	Only use untreated microdilution trays for this antimicrobial agent.[2]
Clindamycin Macrolides Ketolides	*S. aureus* ATCC® 29213 *E. faecalis* ATCC® 29212	MIC too high	pH of media too low	Acceptable pH range = 7.2–7.4 Avoid CO_2 incubation, which lowers pH.
Clindamycin Macrolides Ketolides	*S. aureus* ATCC® 29213 *E. faecalis* ATCC® 29212	MIC too low	pH of media too high	Acceptable pH range = 7.2–7.4
Daptomycin	*S. aureus* ATCC® 29213 *E. faecalis* ATCC® 29212	MICs too high MICs too low	Ca++ content too low Ca++ content too high	Acceptable Ca++ content 50 µg/mL in CAMHB

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Table 5G. (Continued)

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
Tetracyclines	Any	MIC too low	pH of media too low	Acceptable pH range = 7.2–7.4
Tetracyclines	Any	MIC too high	pH of media too high	Acceptable pH range = 7.2–7.4 Avoid CO_2 incubation, which lowers pH.
Tetracyclines	Any	MIC too high	Ca++ and/or Mg++ content too high	Acceptable range = Ca++ 20–25 mg/L Mg++ 10–12.5 mg/L
Tetracyclines	Any	MIC too low	Ca++ and/or Mg++ content too low	Acceptable range = Ca++ 20–25 mg/L Mg++ 10–12.5 mg/L
Omadacycline Tigecycline	Any	MIC too high	CAMHB has not been freshly prepared.	Reference panels must be used or frozen within 12 hours of CAMHB preparation.
ALL AGENTS				
Various	*E. coli* ATCC® 35218 *K. pneumoniae* ATCC® 700603	MIC too low	Spontaneous loss of the plasmid encoding the β-lactamase	See general comment (1) on QC organism maintenance.
Various	Any	One QC result is out of range, but the antimicrobial agent is not an agent reported for patient results (eg, not on hospital formulary).	N/A	If antimicrobial agent is not normally reported, no repeat is necessary if adequate controls are in place to prevent reporting of the out-of-range antimicrobial agent.
Various	Any	Many MICs too low	Inoculum too light; error in inoculum preparation	Repeat using McFarland 0.5 turbidity standard or standardizing device. Check expiration date and proper storage if using barium sulfate or latex standards. Check steps in inoculum preparation and inoculation procedure. Perform colony count check of growth control well immediately after inoculation and before incubation (*E. coli* ATCC® 25922 closely approximates 5 $\times 10^5$ CFU/mL; see M07,[1] Subchapter 3.8).
Various	Any	Many MICs too high or too low	CAMHB not optimal	Use alternative lot.
Various	Any	Many MICs too high or too low	Possible reading/transcription error	Recheck readings. Use alternative lot.
Various	Any	Many MICs too high	Inoculum too heavy	Repeat using McFarland 0.5 turbidity standard or standardizing device. Check expiration date and proper storage if using barium sulfate or latex standards. Check steps in inoculum preparation and inoculation procedure. Perform colony count check of growth control well immediately after inoculation and before incubation (*E. coli* ATCC® 25922 closely approximates 5 $\times 10^5$ CFU/mL; see M07,[1] Subchapter 3.8).

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Table 5G. (Continued)

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
Various	Any	Skipped wells	Contamination. Improper inoculation of panel or inadequate mixing of inoculum. Actual concentration of drug in wells inaccurate. Volume of broth in wells inaccurate.	Repeat QC test. Use alternative lot.
Various	Any	One QC strain is out of range, but other QC strains are in range with the same antimicrobial agent.	One QC organism may be a better indicator of a QC problem (eg, *P. aeruginosa* ATCC® 27853 is a better indicator of imipenem deterioration than *E. coli* ATCC® 25922).	Determine if the in-range QC strain has an on-scale end point for the agent in question. Retest this strain to confirm reproducibility of acceptable results. Evaluate with alternative strains with known MICs. Initiate corrective action with problem QC strain/antimicrobial agent(s).
Various	Any	Two QC strains are out of range with the same antimicrobial agent.	Indicates a problem with the antimicrobial agent. May be a systemic problem.	Initiate corrective action.
Various	Any	One QC result is out of range, but the antimicrobial agent is not an agent reported for patient results (eg, not on hospital formulary).		If antimicrobial agent is not normally reported, no repeat is necessary if adequate controls are in place to prevent reporting of the out-of-range antimicrobial agent. Carefully check antimicrobial agents of the same class for similar trend toward out-of-control results. If the antimicrobial agent in question is consistently out of control, contact the manufacturer.
Various	*E. coli* ATCC® 35218 *K. pneumoniae* ATCC® 700603	MIC to low	Spontaneous loss of the plasmid encoding the β-lactamase	See general comment (1) on QC organism maintenance.

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; CFU, colony-forming unit(s); ESBL, extended-spectrum β-lactamase; MIC, minimal inhibitory concentration; N/A, not applicable; QC, quality control.

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

References for Table 5G

1 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

2 Arhin FF, Sarmiento I, Belley A, et al. Effect of polysorbate 80 on oritavancin binding to plastic surfaces: implications for susceptibility testing. *Antimicrob Agents Chemother.* 2008;52(5):1597-1603.

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Table 6A. Solvents and Diluents for Preparation of Stock Solutions of Antimicrobial Agents[e]

Antimicrobial Agent	Solvent	Diluent
	Unless otherwise stated, use a minimum amount of the listed solvent to solubilize the antimicrobial powder.	**Finish diluting the final stock solution as stated below.**
Amikacin	Water	Water
Amoxicillin	Phosphate buffer, pH 6, 0.1 mol/L	Phosphate buffer, pH 6, 0.1 mol/L
Ampicillin	Phosphate buffer, pH 8, 0.1 mol/L	Phosphate buffer, pH 6, 0.1 mol/L
Avibactam	Water	Water
Azithromycin	95% ethanol or glacial acetic acid[e,f]	Broth media
Azlocillin	Water	Water
Aztreonam	Saturated solution sodium bicarbonate	Water
Besifloxacin	Methanol	Water
Biapenem	Saline[m]	Saline[m]
Cadazolid	DMSO[e]	Water or broth
Carbenicillin	Water	Water
Cefaclor	Water	Water
Cefadroxil	Phosphate buffer, pH 6, 0.1 mol/L	Water
Cefamandole	Water	Water
Cefazolin	Phosphate buffer, pH 6, 0.1 mol/L	Phosphate buffer, pH 6, 0.1 mol/L
Cefdinir	Phosphate buffer, pH 6, 0.1 mol/L	Water
Cefditoren	Phosphate buffer, pH 6, 0.1 mol/L	Water
Cefepime	Phosphate buffer, pH 6, 0.1 mol/L	Phosphate buffer, pH 6, 0.1 mol/L
Cefetamet	Phosphate buffer, pH 6, 0.1 mol/L	Water
Cefiderocol	**Saline[m]**	**Saline[m]**
Cefixime	Phosphate buffer, pH 7, 0.1 mol/L	Phosphate buffer, pH 7, 0.1 mol/L
Cefmetazole	Water	Water
Cefonicid	Water	Water
Cefoperazone	Water	Water
Cefotaxime	Water	Water
Cefotetan	DMSO[e]	Water
Cefoxitin	Water	Water
Cefpodoxime	0.10% (11.9 mmol/L) aqueous sodium bicarbonate	Water
Cefprozil	Water	Water
Ceftaroline	DMSO[e] to 30% of total volume	Saline[m]
Ceftazidime	Sodium carbonate[d]	Water
Ceftibuten	1/10 volume of DMSO[e]	Water
Ceftizoxime	Water	Water
Ceftobiprole	DMSO plus glacial acetic acid[e,h]	Water, vortex vigorously

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Table 6A. (Continued)

Antimicrobial Agent	Solvent	Diluent
	Unless otherwise stated, use a minimum amount of the listed solvent to solubilize the antimicrobial powder.	**Finish diluting the final stock solution as stated below.**
Ceftolozane	Water or saline[m]	Water or saline[m]
Ceftriaxone	Water	Water
Cefuroxime	Phosphate buffer, pH 6, 0.1 mol/L	Phosphate buffer, pH 6, 0.1 mol/L
Cephalexin	Phosphate buffer, pH 6, 0.1 mol/L	Water
Cephalothin	Phosphate buffer, pH 6, 0.1 mol/L	Water
Cephapirin	Phosphate buffer, pH 6, 0.1 mol/L	Water
Cephradine	Phosphate buffer, pH 6, 0.1 mol/L	Water
Chloramphenicol	95% ethanol	Water
Cinoxacin	1/2 volume of water, then add 1 mol/L NaOH dropwise to dissolve	Water
Ciprofloxacin	Water	Water
Clarithromycin	Methanol[e] or glacial acetic acid[e,f]	Phosphate buffer, pH 6.5, 0.1 mol/L
Clavulanate	Phosphate buffer, pH 6, 0.1 mol/L	Phosphate buffer, pH 6, 0.1 mol/L
Clinafloxacin	Water	Water
Clindamycin	Water	Water
Colistin[a]	Water	Water
Dalbavancin	DMSO[e]	DMSO[e,g]
Daptomycin	Water	Water
Delafloxacin	1/2 volume of water, then 0.1 mol/L NaOH dropwise to dissolve	Water
Dirithromycin	Glacial acetic acid[f]	Water
Doripenem	Saline[m]	Saline[m]
Doxycycline	Water	Water
Enoxacin	1/2 volume of water, then 0.1 mol/L NaOH dropwise to dissolve	Water
Eravacycline	Water	Water
Ertapenem	Phosphate buffer, pH 7.2, 0.01 mol/L	Phosphate buffer, pH 7.2, 0.01 mol/L
Erythromycin	95% ethanol or glacial acetic acid[e,f]	Water
Faropenem	Water	Water
Fidaxomicin	DMSO[e]	Water
Finafloxacin	Water	Water
Fleroxacin	1/2 volume of water, then 0.1 mol/L NaOH dropwise to dissolve	Water
Fosfomycin	Water	Water
Fusidic acid	Water	Water
Garenoxacin	Water (with stirring)	Water

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Table 6A. (Continued)

Antimicrobial Agent	Solvent	Diluent
	Unless otherwise stated, use a minimum amount of the listed solvent to solubilize the antimicrobial powder.	**Finish diluting the final stock solution as stated below.**
Gatifloxacin	Water (with stirring)	Water
Gemifloxacin	Water	Water
Gentamicin	Water	Water
Gepotidacin	DMSO[e]	Water
Iclaprim	DMSO[e]	Water
Imipenem	Phosphate buffer, pH 7.2, 0.01 mol/L	Phosphate buffer, pH 7.2, 0.01 mol/L
Kanamycin	Water	Water
Lefamulin	Water	Water
Levofloxacin	1/2 volume of water, then 0.1 mol/L NaOH dropwise to dissolve	Water
Levonadifloxacin	27.5 μg/mL solution of L-arginine in water	Water
Linezolid	Water	Water
Lomefloxacin	Water	Water
Loracarbef	Water	Water
Mecillinam	Water	Water
Meropenem	Water	Water
Meropenem-vaborbactam	DMSO[e]	Water
Methicillin	Water	Water
Metronidazole	DMSO[e]	Water
Mezlocillin	Water	Water
Minocycline	Water	Water
Moxalactam (diammonium salt)[b]	0.04 mol/L HCl (let sit for 1.5 to 2 hours)	Phosphate buffer, pH 6, 0.1 mol/L
Moxifloxacin	Water	Water
Mupirocin	Water	Water
Nafcillin	Water	Water
Nafithromycin	½ volume of water, then glacial acetic acid dropwise to dissolve (acetic acid not to exceed 2.5 μL/mL).	Water
Nalidixic acid	1/2 volume of water, then add 1 mol/L NaOH dropwise to dissolve	
Netilmicin	Water	Water
Nitazoxanide	DMSO[e,l]	DMSO[e,l]
Nitrofurantoin[c]	Phosphate buffer, pH 8, 0.1 mol/L	Phosphate buffer, pH 8, 0.1 mol/L
Norfloxacin	1/2 volume of water, then 0.1 mol/L NaOH dropwise to dissolve	Water

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Table 6A. (Continued)

Antimicrobial Agent	Solvent	Diluent
	Unless otherwise stated, use a minimum amount of the listed solvent to solubilize the antimicrobial powder.	**Finish diluting the final stock solution as stated below.**
Ofloxacin	1/2 volume of water, then 0.1 mol/L NaOH dropwise to dissolve	Water
Omadacycline	Water	Water
Oritavancin	0.002% polysorbate-80 in water[i]	0.002% polysorbate-80 in water[i]
Oxacillin	Water	Water
Penicillin	Water	Water
Pexiganan	Water	Water
Piperacillin	Water	Water
Plazomicin	Water	Water
Polymyxin B	Water	Water
Quinupristin-dalfopristin	Water	Water
Ramoplanin	Water	Water
Razupenem	Phosphate buffer, pH 7.2, 0.01 mol/L	Phosphate buffer, pH 7.2, 0.01 mol/L
Relebactam	Water	Water
Ridinilazole	**DMSO[e]**	**DMSO[e]**
Rifampin	Methanol[e] (maximum concentration = 640 μg/mL)	Water (with stirring)
Rifaximin	Methanol[e]	0.1 M phosphate buffer, pH 7.4 + 0.45% sodium dodecyl sulfonate
Secnidazole	DMSO[e]	Water
Solithromycin	Glacial acetic acid[f]	Water
Sparfloxacin	Water	Water
Spectinomycin	Water	Water
Streptomycin	Water	Water
Sulbactam	Water	Water
Sulfonamides	1/2 volume hot water and minimal amount of 2.5 mol/L NaOH to dissolve	Water
Sulopenem[j]	0.01 M phosphate buffer, pH 7.2, vortex to dissolve	0.01 M phosphate buffer, pH 7.2
Surotomycin	Water	Water
Tazobactam	Water	Water
Tedizolid	DMSO[e]	DMSO[e,n]
Teicoplanin	Water	Water
Telavancin	DMSO[e]	DMSO[e,g]
Telithromycin	Glacial acetic acid[e,f]	Water
Tetracycline	Water	Water
Ticarcillin	Phosphate buffer, pH 6, 0.1 mol/L	Phosphate buffer, pH 6, 0.1 mol/L
Ticarcillin-clavulanate	Phosphate buffer, pH 6, 0.1 mol/L	Phosphate buffer, pH 6, 0.1 mol/L

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Table 6A. (Continued)

Antimicrobial Agent	Solvent	Diluent
	Unless otherwise stated, use a minimum amount of the listed solvent to solubilize the antimicrobial powder.	**Finish diluting the final stock solution as stated below.**
Tigecycline	Water	Water
Tinidazole	DMSO[e,l]	Water
Tizoxanide	DMSO[e,l]	DMSO[e,l]
Tobramycin	Water	Water
Trimethoprim	0.05 mol/L lactic[e] or hydrochloric[e] acid, 10% of final volume	Water (may need heat)
Trimethoprim (if lactate)	Water	Water
Trospectomycin	Water	Water
Ulifloxacin (prulifloxacin)	DMSO[e]	Water
Vaborbactam	**90% DMSO[e]/10% water**	**Water**
Vancomycin	Water	Water

Abbreviation: DMSO, dimethyl sulfoxide.

Footnotes

a. The formulation of colistin reference standard powder used in antimicrobial susceptibility tests is colistin sulfate and not colistin methane sulfonate (sulfomethate).

b. The diammonium salt of moxalactam is very stable, but it is almost pure R isomer. Moxalactam for clinical use is a 1:1 mixture of R and S isomers. Therefore, the salt is dissolved in 0.04 mol/L HCl and allowed to react for 1.5 to 2 hours to convert it to equal parts of both isomers.

c. Alternatively, nitrofurantoin is dissolved in DMSO.

d. Anhydrous sodium carbonate is used at a weight of exactly 10% of the ceftazidime to be used. The sodium carbonate is dissolved in solution in most of the necessary water. The antimicrobial agent is dissolved in this sodium carbonate solution, and water is added to the desired volume. The solution is to be used as soon as possible, but it can be stored up to six hours at no more than 25°C.

e. Consult the safety data sheets before working with any antimicrobial reference standard powder, solvent, or diluent. Some of the compounds (eg, solvents such as DMSO, methanol) are more toxic than others and may necessitate handling in a chemical fume hood.

f. For glacial acetic acid, use 1/2 volume of water, then add glacial acetic acid dropwise until dissolved, not to exceed 2.5 µL/mL.

g. Starting stock solutions of dalbavancin and telavancin should be prepared at concentrations no higher than 1600 µg/mL. Intermediate 100× concentrations should then be diluted in DMSO. Final 1:100 dilutions should then be made directly into cation-adjusted Mueller-Hinton broth (CAMHB) supplemented with 0.002% (v/v) polysorbate-80, so the final concentration of DMSO in the wells is no greater than 1%. See also Table 8B.

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Table 6A. (Continued)

h. For each 1.5 mg of ceftobiprole, add 110 μL of a 10:1 mixture of DMSO and glacial acetic acid. Vortex vigorously for one minute, then intermittently for 15 minutes. Dilute to 1 mL with distilled water.

i. Starting stock solutions of oritavancin should be prepared at concentrations no higher than 1600 μg/mL in 0.002% polysorbate-80 in water. Intermediate 100× oritavancin concentrations should then be prepared in 0.002% polysorbate-80 in water. Final 1:100 dilutions should be made directly into CAMHB supplemented with 0.002% polysorbate-80, so the final concentration of polysorbate-80 in the wells is 0.002%.

j. Must be made fresh on the day of use.

k. Dimethylformamide to 25% of final volume/water.

l. Final concentration of DMSO should not exceed 1%. This may be accomplished as follows: 1) prepare the stock solution at 10 times higher concentration than planned stock solution (ie, prepare at 12 800 μg/mL, rather than 1280 μg/mL); 2) add 1.8 mL sterile water to each agar deep; 3) add 0.2 mL of each antibiotic dilution to each agar deep.

m. Saline – a solution of 0.85% to 0.9% NaCl (w/v).

n. Starting stock solutions of tedizolid should be prepared at concentrations no higher than 1600 μg/mL. Intermediate 100× concentrations should be diluted in DMSO. Final 1:100 dilutions should be made directly into CAMHB, so that the final concentration of DMSO in the wells is no greater than 1%. See also Table 8B.

NOTE: Information in boldface type is new or modified since the previous edition.

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Table 6B. Preparation of Stock Solutions for Antimicrobial Agents Provided With Activity Expressed as Units

Antimicrobial Agent	Pure Agent (Reference)	Calculation for µg/mg	Example
Potassium Penicillin G	0.625 µg/unit[1]	Multiply the activity expressed in units/mg by 0.625 µg/unit.	Activity units/mg • 0.625 µg/unit = Activity µg/mg (eg, 1592 units/mg • 0.625 µg/unit = 995 µg/mg)
Sodium Penicillin G	0.6 µg/unit[1]	Multiply the activity expressed in units/mg by 0.6 µg/unit.	Activity units/mg • 0.6 µg/unit = Activity µg/mg (eg, 1477 units/mg • 0.6 µg/unit = 886.2 µg/mg)
Polymyxin B	10 000 units/mg = 10 units/µg = 0.1 µg/unit[2]	Multiply the activity expressed in units/mg by 0.1 µg/unit.	Activity units/mg • 0.1 µg/unit = Activity µg/mg (eg, 8120 units/mg • 0.1 µg/unit = 812 µg/mg)
		Divide the activity expressed in units/mg by 10 units/µg.	Activity units/mg / 10 units/µg = Activity µg/mg (eg, 8120 units/mg / 10 units/mg = 812 µg/mg)
Colistin sulfate[a]	30 000 units/mg = 30 units/µg = 0.03333 µg/unit[2]	Multiply the activity expressed in units/mg by 0.03333 µg/unit.	Activity units/mg • 0.03333 µg/unit = Activity µg/mg (eg, 20 277 units/mg • 0.03333 µg/unit = 676 µg/mg)
		Divide the activity expressed in units/mg by 30 units/mg.	Activity units/mg / 30 units/µg = Activity µg/mg (eg, 20 277 units/mg / 30 units/µg = 676 µg/mg)
Streptomycin	785 units/mg[3]	Divide the number of units given for the powder by 785. This gives the percent purity of the powder. Multiply the percent purity by 850, which is the amount in the purest form of streptomycin. This result equals the activity factor in µg/mg.	([Potency units/mg] / [785 units/mg]) • (850 µg/mg) = Potency µg/mg (eg, [751 units/mg / 785 units/mg] • 850 µg/mg = 813 µg/mg) If powder contains 2.8% water: 813 • (1 – 0.028) = potency 813 • 0.972 = 790 µg/mg

Footnote

a. Do not use colistin methanesulfonate for *in vitro* antimicrobial susceptibility tests.

References for Table 6B

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Table 6C. Preparing Solutions and Media Containing Combinations of Antimicrobial Agents

Antimicrobial Agent	Combination Tested	Preparation	Example
Amikacin-fosfomycin	5:2 ratio (amikacin:fosfomycin)	Prepare 10× starting concentration as 5:2 ratio and dilute as needed. **NOTE:** Media should be supplemented with 25 μg/mL of glucose-6-phosphate.	
Amoxicillin-clavulanate	2:1 ratio (amoxicillin:clavulanate)	Prepare 10× starting concentration as 2:1 ratio and dilute as needed.	For a starting concentration of 128/64 in the panel, prepare a 10× stock concentration of 2560 μg/mL for amoxicillin and 1280 μg/mL for clavulanate. Then combine equal amounts of each to the first dilution tube, which will then contain 1280/640 μg/mL of the combination. Dilute 1:10 with broth to achieve the final concentration in microdilution wells.
Ampicillin-sulbactam	2:1 ratio (ampicillin:sulbactam)	Same as amoxicillin-clavulanate.	
Aztreonam-avibactam	Fixed concentration of avibactam at 4 μg/mL	Prepare 10× starting concentration of aztreonam at twice the concentration needed and dilute as usual using serial twofold dilutions. Add an equal volume of avibactam 80 μg/mL to each of the diluted tubes.	For a starting concentration of 128/4 in the panel, prepare a 10× stock concentration of aztreonam at 2560 μg/mL and dilute by serial twofold increments down to the final concentration needed in the panel. Prepare a stock concentration of avibactam at 80 μg/mL. Then add an equal volume of the avibactam 80 μg/mL solution to each diluted tube of aztreonam. For example, 5 mL of 2560 μg/mL aztreonam + 5 mL of 80 μg/mL avibactam = 10 mL of 1280/40 μg/mL aztreonam-avibactam. Dilute 1:10 with broth to achieve the final concentration in microdilution wells.
Cefepime-tazobactam	Fixed concentration of tazobactam at 8 μg/mL	Prepare 10× starting concentration of cefepime at twice the concentration needed and dilute as usual using serial twofold dilutions. Add an equal volume of tazobactam 160 μg/mL to each of the diluted tubes.	For a starting concentration of 128/8 in the panel, prepare a 10× stock concentration of cefepime at 2560 μg/mL and dilute by serial twofold increments down to the final concentration needed in the panel. Prepare a stock concentration of tazobactam at 160 μg/mL. Then add an equal volume of the tazobactam 160 μg/mL solution to each diluted tube of cefepime. For example, 5 mL of 2560 μg/mL cefepime + 5 mL of 160 μg/mL tazobactam = 10 mL of 1280/80 μg/mL cefepime-tazobactam. Dilute 1:10 with broth to achieve the final concentration in the microdilution wells.
Cefepime-zidebactam	**1:1 ratio (cefepime:zidebactam)**	**Prepare 10× starting concentration as 1:1 ratio and dilute as needed.**	**For a starting concentration of 128/128 in the panel, prepare a 20× stock concentration of 2560 μg/mL for cefepime and 2560 μg/mL for zidebactam. Then combine equal amounts of each to the first dilution tube, which will then contain 1280/1280 μg/mL of the combination. Prepare twofold serial dilutions and dilute each 1:10 with broth to achieve the final concentration in the microdilution wells.**
Ceftaroline-avibactam	Fixed concentration of avibactam at 4 μg/mL	Same as aztreonam-avibactam.	
Ceftazidime-avibactam	Fixed concentration of avibactam at 4 μg/mL	Same as aztreonam-avibactam.	

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Table 6C. (Continued)

Antimicrobial Agent	Combination Tested	Preparation	Example
Ceftolozane-tazobactam	Fixed concentration of tazobactam at 4 µg/mL	Same as aztreonam-avibactam.	
Imipenem-relebactam	Fixed concentration of relebactam at 4 µg/mL	Same as aztreonam-avibactam.	
Meropenem-vaborbactam	**Fixed concentration of vaborbactam at 8 µg/mL**	**Prepare 10× starting concentration of meropenem at twice the concentration needed and dilute as usual using serial twofold dilutions. Add an equal volume of vaborbactam 160 µg/mL to each of the diluted tubes.**	**For a starting concentration of 64/8 µg/mL in the panel, prepare a 10× stock concentration of meropenem at 1280 µg/mL and dilute by serial twofold increments down to the final concentration needed in the panel. Prepare a stock concentration of vaborbactam at 160 µg/mL. Then add an equal volume of the vaborbactam 160 µg/mL solution to each diluted tube of meropenem. For example, 5 mL of 1280 µg/mL meropenem + 5 mL of 160 µg/mL vaborbactam = 10 mL of 640/80 µg/mL meropenem-vaborbactam. Dilute 1:10 with broth to achieve the final concentration in the microdilution wells.**
Piperacillin-tazobactam	Fixed concentration of tazobactam at 4 µg/mL	Same as aztreonam-avibactam.	
Ticarcillin-clavulanate	Fixed concentration of clavulanate at 2 µg/mL	Prepare 10× starting concentration of ticarcillin at twice the concentration needed and dilute as usual using serial twofold dilutions. Add an equal volume of clavulanate 40 µg/mL to each of the diluted tubes.	For a starting concentration of 128/2 in the panel, prepare a 10× stock concentration of ticarcillin at 2560 µg/mL and dilute by serial twofold increments down to the final concentration needed. Prepare a stock concentration of clavulanate at 40 µg/mL. Then add an equal volume of the clavulanate 40 µg/mL solution to each diluted tube of ticarcillin. For example, 5 mL of 2560 µg/mL ticarcillin + 5 mL of 40 µg/mL clavulanate = 10 mL of 1280/20 µg/mL ticarcillin-clavulanate. Dilute 1:10 with broth to achieve the final concentration in microdilution wells.
Trimethoprim-sulfamethoxazole	1:19 ratio (trimethoprim:sulfamethoxazole)	Prepare a 10× starting concentration of trimethoprim at 1600 µg/mL (or at 1280 µg/mL that will need dilution to 160 µg/mL). Prepare a 10× starting concentration of sulfamethoxazole at a $\log_2$ multiple of 1520 µg/mL (eg, 1520, 3040, or 6080 µg/mL) depending on the starting concentration needed.	For a starting concentration of 8/152 in the panel, prepare a 10× concentration of trimethoprim at 160 µg/mL. Prepare a 10× starting concentration of sulfamethoxazole at 3040 µg/mL. Add an equal volume of the 160 µg/mL trimethoprim and the 3040 µg/mL sulfamethoxazole to the first dilution tube, and then dilute by serial twofold dilutions as usual. For example, 5 mL of 160 µg/mL trimethoprim and 5 mL of 3040 µg/mL sulfamethoxazole = 10 mL of 80/1520 trimethoprim-sulfamethoxazole. Dilute 1:10 with broth to achieve the final concentration in microdilution wells.
Quinupristin-dalfopristin	Preparation usually not necessary, because drug powder is received as combination.		

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Table 6C. (Continued)

NOTE 1: To prepare intermediate dilutions of antimicrobial agents, a convenient formula to use is $C_1 \cdot V_1 = C_2 \cdot V_2$, where C_1 is the concentration of stock solution of the antimicrobial agent (usually 1280 µg/mL or greater); V_1 is the unknown volume that will be needed to make the intermediate concentration; C_2 is the intermediate concentration needed; and V_2 is the volume of the intermediate stock solution needed.

NOTE 2: Information in boldface type is new or modified since the previous edition.

For example: To prepare 20 mL of a 40 µg/mL solution from a 1280 µg/mL stock solution:

$$C_1 \cdot V_1 = C_2 \cdot V_2$$

$$1280\ \mu g/mL \cdot V_1 = 40\ \mu g/mL \cdot 20\ mL$$

$$V_1 = \frac{40\ \mu g/mL \cdot 20\ mL}{1280\ \mu g/mL}$$

$$V_1 = 0.625\ mL$$

Therefore, add 0.625 mL of the 1280 µg/mL stock solution to 19.375 mL of diluent (usually water) for a final volume of 20 mL of a 40 µg/mL solution.

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Table 7. Preparing Dilutions of Antimicrobial Agents to Be Used in Agar Dilution Susceptibility Tests

Antimicrobial Solution										
Step	Concentration, µg/mL	Source	Volume, mL	+	Diluent, mL	=	Intermediate Concentration, µg/mL	=	Final Concentration at 1:10 Dilution in Agar, µg/mL	Log_2
	5120	Stock	–		–		5120		512	9
1	5120	Stock	2		2		2560		256	8
2	5120	Stock	1		3		1280		128	7
3	5120	Stock	1		7		640		64	6
4	640	Step 3	2		2		320		32	5
5	640	Step 3	1		3		160		16	4
6	640	Step 3	1		7		80		8	3
7	80	Step 6	2		2		40		4	2
8	80	Step 6	1		3		20		2	1
9	80	Step 6	1		7		10		1	0
10	10	Step 9	2		2		5		0.5	−1
11	10	Step 9	1		3		2.5		0.25	−2
12	10	Step 9	1		7		1.25		0.125	−3

NOTE: This table is modified from Ericsson HM, Sherris JC. Antibiotic sensitivity testing: report of an international collaborative study. *Acta Pathol Microbiol Scand B Microbiol Immunol*. 1971;217(suppl):1+.

When serial twofold dilution minimal inhibitory concentrations are being prepared and tested, the actual dilution scheme is:

128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.0078125, 0.0039063, 0.0019531 µg/mL, etc.

For convenience only, and not because these are the actual concentrations tested, it was decided to use the following values in these tables:

128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.12, 0.06, 0.03, 0.016, 0.008, 0.004, 0.002 µg/mL, etc.

The values that appear in the tables are equivalent to the actual values tested, eg, 0.12 µg/mL = 0.125 µg/mL, 0.016 µg/mL = 0.015625 µg/mL.

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Table 8A. Preparing Dilutions of Antimicrobial Agents to Be Used in Broth Dilution Susceptibility Tests

Antimicrobial Solution								
Step	Concentration,[a] µg/mL	Source	Volume,[a] mL	+	CAMHB[b] Volume,[c] mL	=	Final Concentration, µg/mL	Log_2
1	5120	Stock	1		9		512	9
2	512	Step 1	1		1		256	8
3	512	Step 1	1		3		128	7
4	512	Step 1	1		7		64	6
5	64	Step 4	1		1		32	5
6	64	Step 4	1		3		16	4
7	64	Step 4	1		7		8	3
8	8	Step 7	1		1		4	2
9	8	Step 7	1		3		2	1
10	8	Step 7	1		7		1	0
11	1	Step 10	1		1		0.5	−1
12	1	Step 10	1		3		0.25	−2
13	1	Step 10	1		7		0.125	−3

Abbreviation: CAMHB, cation-adjusted Mueller-Hinton broth.

NOTE: This table is modified from Ericsson HM, Sherris JC. Antibiotic sensitivity testing: report of an international collaborative study. *Acta Pathol Microbiol Scand B Microbiol Immunol*. 1971;217(suppl):1:+.

Footnotes

a. See Table 7 for the actual dilution scheme when serial twofold dilution minimal inhibitory concentrations are being prepared and tested.

b. Adjustment with cations, if necessary, occurs before this step.

c. The volumes selected can be any multiple of these figures, depending on the number of tests to be performed.

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Table 8B. Preparing Dilutions of Water-Insoluble Antimicrobial Agents to Be Used in Broth Dilution Susceptibility Tests

Antimicrobial Solution											
Step	Concentration, µg/mL	Source	Volume, mL	+	Solvent, mL (eg, DMSO)	=	Intermediate Concentration, µg/mL	=	Final Concentration at 1:100, µg/mL	Log_2
1	1600	Stock					1600		16	4
2	1600	Stock	0.5		0.5		800		8.0	3
3	1600	Stock	0.5		1.5		400		4.0	2
4	1600	Stock	0.5		3.5		200		2.0	1
5	200	Step 4	0.5		0.5		100		1.0	0
6	200	Step 4	0.5		1.5		50		0.5	−1
7	200	Step 4	0.5		3.5		25		0.25	−2
8	25	Step 7	0.5		0.5		12.5		0.125	−3
9	25	Step 7	0.5		1.5		6.25		0.0625	−4
10	25	Step 7	0.5		3.5		3.1		0.03	−5
11	3.1	Step 10	0.5		0.5		1.6		0.015	−6
12	3.1	Step 10	0.5		1.5		0.8		0.008	−7
13	3.1	Step 10	0.5		3.5		0.4		0.004	−8
14	0.4	Step 13	0.5		0.5		0.2		0.002	−9

Abbreviation: DMSO, dimethyl sulfoxide.

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Appendix A. Suggestions for Confirming Resistant, Intermediate, or Nonsusceptible Antimicrobial Susceptibility Test Results and Organism Identification

Organism or Organism Group	Resistance Phenotype Detected[a]	Occurrence and Significance of Resistance and Actions to Take Following Confirmation of Results[a]		
		Category I	**Category II**	**Category III**
		Not reported or only rarely reported to date	**Uncommon in most institutions**	**May be common, but is generally considered of epidemiological concern**
		Action Steps:		
		• Confirm ID and susceptibility.[a] • Report to infection control. • Send to public health laboratory. • Save isolate. **NOTE:** It may be appropriate to notify infection control of preliminary findings before confirmation of results.	• Confirm ID and susceptibility if uncommon in the institution.[a] • Check with infection control in the facility to determine if special reporting procedures or additional action are needed. • Check with local public health department to determine which isolates should be reported to them and when isolates should be sent to the public health laboratory.	• Confirm ID and susceptibility if uncommon in the institution.[a] • Check with infection control in facility to determine if special reporting procedures or additional action are needed.
Any *Enterobacteriaceae*	Carbapenem – I or R[b]		x	
	Colistin/Polymyxin – **NWT**	x		
	Amikacin, gentamicin, and tobramycin – R			x
Escherichia coli *Klebsiella* spp. *Proteus mirabilis*	Extended-spectrum cephalosporin[c] – I or R			x
Salmonella and *Shigella* spp.[d]	Cephalosporin III – I or R Fluoroquinolone – I or R		x	
Acinetobacter baumannii	Colistin/polymyxin – R		x	
	Carbapenem – I or R			x
Pseudomonas aeruginosa	Colistin– R		x	
	Polymyxin – I or R		x	
	Amikacin, gentamicin, and tobramycin – R Carbapenem – I or R			x

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Appendix A. (Continued)

Organism or Organism Group	Resistance Phenotype Detected[a]	Occurrence and Significance of Resistance and Actions to Take Following Confirmation of Results[a]		
		Category I	Category II	Category III
		Not reported or only rarely reported to date	Uncommon in most institutions	May be common, but is generally considered of epidemiological concern
Stenotrophomonas maltophilia	Trimethoprim-sulfamethoxazole – I or R		x	
Haemophilus influenzae	Carbapenem – NS Ceftaroline – NS Extended-spectrum cephalosporin[c] – NS Fluoroquinolone – NS	x		
	Amoxicillin-clavulanate – R Ampicillin – R and β-lactamase negative		x	
Neisseria gonorrhoeae	Extended-spectrum cephalosporin[c] – NS		x	
	Fluoroquinolone – I or R			x
Neisseria meningitidis	Ampicillin or penicillin – R Extended-spectrum cephalosporin[c] – NS Meropenem – NS	x		
	Ampicillin or penicillin – I Azithromycin – NS Chloramphenicol – I or R Fluoroquinolone – I or R Minocycline – NS Rifampin – I or R		x	
Enterococcus spp.	**Dalbavancin – NS** Oritavancin – NS Telavancin – NS	x		
	Daptomycin – NS Linezolid – R Tedizolid – NS		x	
	High-level aminoglycoside – R Vancomycin – R			x

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Appendix A. (Continued)

Organism or Organism Group	Resistance Phenotype Detected[a]	Occurrence and Significance of Resistance and Actions to Take Following Confirmation of Results[a]		
		Category I	Category II	Category III
		Not reported or only rarely reported to date	Uncommon in most institutions	May be common, but is generally considered of epidemiological concern
Staphylococcus aureus	**Dalbavancin – NS** Oritavancin – NS Telavancin – NS	x		
	Vancomycin MIC ≥ 8 µg/mL[e]		x[e]	
	Ceftaroline – R Daptomycin – NS Linezolid – R Quinupristin-dalfopristin – I or R Tedizolid – R Vancomycin MIC = 4 µg/mL		x	
	Oxacillin – R			x
Coagulase-negative staphylococci	Daptomycin – NS Linezolid – R Quinupristin-dalfopristin – I or R Vancomycin – I or R[f]		x	
Streptococcus pneumoniae	Ceftaroline – NS Linezolid – NS Vancomycin – NS	x		
	Fluoroquinolone – I or R Imipenem or meropenem – I or R Quinupristin-dalfopristin – I or R Rifampin – I or R		x	
	Using nonmeningitis breakpoints: Amoxicillin or penicillin – R Extended-spectrum cephalosporin[c] – R			x

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Appendix A. (Continued)

Organism or Organism Group	Resistance Phenotype Detected[a]	Occurrence and Significance of Resistance and Actions to Take Following Confirmation of Results[a]		
		Category I	Category II	Category III
		Not reported or only rarely reported to date	Uncommon in most institutions	May be common, but is generally considered of epidemiological concern
Streptococcus, β-hemolytic group[g]	Ampicillin or penicillin – NS Ceftaroline – NS **Dalbavancin – NS** Daptomycin – NS Ertapenem or meropenem – NS Extended-spectrum cephalosporin[c] – NS Linezolid – NS Oritavancin – NS Tedizolid – NS Telavancin – NS Vancomycin – NS	x		
	Quinupristin-dalfopristin – I or R		x	
Streptococcus, viridans group	**Dalbavancin – NS** Daptomycin – NS Ertapenem or meropenem – NS Linezolid – NS Oritavancin – NS Quinupristin-dalfopristin – I or R Tedizolid – NS Telavancin – NS Vancomycin – NS	x		
Bacteroides fragilis group	Metronidazole – I or R		x	
	Doripenem, ertapenem, imipenem, or meropenem – I or R		x	

Abbreviations: I, intermediate; ID, identification; MIC, minimal inhibitory concentration; NS, nonsusceptible; NWT, non-wild-type; R, resistant.

NOTE 1: NS: A category used for isolates for which only a susceptible interpretive criterion has been designated because of the absence or rare occurrence of resistant strains. Isolates that have MICs above or zone diameters below the value indicated for the susceptible breakpoint should be reported as nonsusceptible.

NOTE 2: An isolate that is interpreted as nonsusceptible does not necessarily mean that the isolate has a resistance mechanism. It is possible that isolates with MICs above the susceptible breakpoint that lack resistance mechanisms may be encountered within the wild-type distribution subsequent to the time the susceptible-only breakpoint is set.

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Appendix A. (Continued)

NOTE 3: For strains yielding results in the "nonsusceptible" category, organism identification and antimicrobial susceptibility test results should be confirmed (see footnote "a").

Footnotes

a. Ensure antimicrobial susceptibility test results and organism identification are accurate and reproducible. Consider the following steps:
   1. Check for transcription errors, contamination, or defective panel, plate, or card.
   2. Check previous reports on the patient to determine if the isolate was encountered and confirmed earlier.
   3. Repeat organism identification and antimicrobial susceptibility tests with initial method to ensure they reproduce. (For category I and II, the laboratory may elect to skip step 3 and go to steps 4 and 5. For category III, repeat and/or confirmatory testing may not be needed if resistance is common in the institution.)
   4. Confirm organism identification with second method performed in-house or at a referral laboratory.
   5. Confirm antimicrobial susceptibility test results with second method (eg, in-house or referral laboratory). The second method might be a CLSI reference method (eg, broth microdilution, agar dilution, or disk diffusion) or a US Food and Drug Administration–cleared commercial test.

b. Imipenem MICs for *Proteus* spp., *Providencia* spp., and *Morganella morganii* tend to be higher (eg, MICs in the intermediate or resistant category first published in June 2010 [M100-S20-U]) than those with meropenem or doripenem MICs. These isolates may have elevated MICs by mechanisms other than production of carbapenemases.

c. Extended-spectrum cephalosporin = cephalosporin III or IV (see Glossary I).

d. When submitting the report to a public health department, include antimicrobial susceptibility test results for *Salmonella* spp. that are intermediate or resistant to third-generation cephalosporins (cephalosporin III) and/or intermediate or resistant to fluoroquinolone or resistant to nalidixic acid.

e. Rarely encountered. Because of significant infection control and public health implications, follow Category I recommendations for notifying infection control and public health authorities.

f. There are some species of coagulase-negative staphylococci (CoNS) for which vancomycin MICs may test within the intermediate range. In contrast, vancomycin-resistant CoNS are rare.

g. Confirm that groups C and G are large colony and not small colony variants. Groups C and G small colony variants are included with the viridans group.

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Appendix B. Intrinsic Resistance

Intrinsic resistance is defined as inherent or innate (not acquired) antimicrobial resistance, which is reflected in wild-type antimicrobial patterns of all or almost all representatives of a species. Intrinsic resistance is so common that susceptibility testing is unnecessary. For example, *Citrobacter* spp. are intrinsically resistant to ampicillin.

These tables can be helpful in at least three ways: 1) they provide a way to evaluate the accuracy of testing methods; 2) they aid in the recognition of common phenotypes; and 3) they can assist with verification of cumulative antimicrobial susceptibility test data. In the tables, an "R" occurring with an antimicrobial agent/organism combination means that strains should test resistant. A small percentage (1% to 3%) may appear susceptible due to method variation, mutation, or low levels of resistance expression.

Each laboratory should decide which agents to test and report in consultation with institutional leaders representing infectious diseases practitioners, the pharmacy and therapeutics and infection control committees of the medical staff, and the antimicrobial stewardship team. If tested, the result for an antimicrobial agent/organism combination listed as having intrinsic resistance should be reported as resistant. Consideration may be given to adding comments regarding intrinsic resistance of agents not tested. See Appendix A, footnote "a."

B1. *Enterobacteriaceae*

Antimicrobial Agent / Organism	Ampicillin	Amoxicillin-clavulanate	Ampicillin-sulbactam	Piperacillin	Ticarcillin	Cephalosporins I: Cefazolin, Cephalothin	Cephamycins: Cefoxitin, Cefotetan	Cephalosporin II: Cefuroxime	Imipenem	Tetracyclines	Tigecycline	Nitrofurantoin	Polymyxin B Colistin	Aminoglycosides
Citrobacter freundii	R	R	R			R	R	R						
Citrobacter koseri	R			R	R									
Enterobacter cloacae complex[a]	R	R	R			R	R	R						
Escherichia coli	There is no intrinsic resistance to β-lactams in this organism.													
Escherichia hermannii	R				R									
Hafnia alvei	R	R	R			R	R							
Klebsiella* (*formerly* *Enterobacter*) *aerogenes	R	R	R			R	R	R						
Klebsiella pneumoniae	R				R									
Morganella morganii	R	R				R		R	[b]		R	R	R	
Proteus mirabilis	There is no intrinsic resistance to penicillins and cephalosporins in this organism.								[b]	R	R	R	R	
Proteus penneri	R					R		R	[b]	R	R	R	R	
Proteus vulgaris	R					R		R	[b]	R	R	R	R	
Providencia rettgeri	R	R				R			[b]	R	R	R	R	
Providencia stuartii	R	R				R			[b]	R	R	R	R	[c]

Appendix B. (Continued)

B1. *Enterobacteriaceae* (Continued)

Organism \ Antimicrobial Agent	Ampicillin	Amoxicillin-clavulanate	Ampicillin-sulbactam	Piperacillin	Ticarcillin	Cephalosporins I: Cefazolin, Cephalothin	Cephamycins: Cefoxitin, Cefotetan	Cephalosporin II: Cefuroxime	Imipenem	Tetracyclines	Tigecycline	Nitrofurantoin	Polymyxin B Colistin	Aminoglycosides
Salmonella and *Shigella* spp.	There is no intrinsic resistance to β-lactams in these organisms; refer to **WARNING** below for reporting.													
Serratia marcescens	R	R	R			R	R	R				R	R	
Yersinia enterocolitica	R	R			R	R								

Abbreviation: R, resistant.

WARNING: For *Salmonella* spp. and *Shigella* spp., aminoglycosides, first- and second-generation cephalosporins, and cephamycins may appear active *in vitro,* but are not effective clinically and should not be reported as susceptible.

NOTE 1: Cephalosporins III, cefepime, aztreonam, ticarcillin-clavulanate, piperacillin-tazobactam, and the carbapenems are not listed, because there is no intrinsic resistance in *Enterobacteriaceae.*

NOTE 2: *Enterobacteriaceae* are also intrinsically resistant to clindamycin, daptomycin, fusidic acid, glycopeptides (vancomycin, teicoplanin), lipoglycopeptides (oritavancin, telavancin), linezolid, tedizolid, quinupristin-dalfopristin, rifampin, and macrolides (erythromycin, clarithromycin, and azithromycin). However, there are some exceptions with macrolides (eg, *Salmonella* and *Shigella* spp. with azithromycin).

NOTE 3: Information in boldface type is new or modified since the previous edition.

Footnotes

a. *E. cloacae* complex includes *Enterobacter asburiae, Enterobacter cloacae,* and *Enterobacter hormaechei*. Other members of the complex include *Enterobacter kobei* and *Enterobacter ludwigii,* for which antimicrobial susceptibility testing data are not available.

b. *Proteus* species, *Providencia* species, and *Morganella* species may have elevated minimal inhibitory concentrations to imipenem by mechanisms other than by production of carbapenemases. Isolates that test as susceptible should be reported as susceptible.

c. *P. stuartii* should be considered resistant to gentamicin, netilmicin, and tobramycin but not intrinsically resistant to amikacin.

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Appendix B. (Continued)

B2. Non-*Enterobacteriaceae*

Organism \ Antimicrobial Agent	Ampicillin, Amoxicillin	Piperacillin	Ticarcillin	Ampicillin-sulbactam	Amoxicillin- clavulanate	Piperacillin-tazobactam	Cefotaxime	Ceftriaxone	Ceftazidime	Cefepime	Aztreonam	Imipenem	Meropenem	Ertapenem	Polymyxin B Colistin	Aminoglycosides	Tetracyclines/ Tigecycline	Trimethoprim	Trimethoprim-sulfamethoxazole	Chloramphenicol	Fosfomycin
Acinetobacter baumannii/ Acinetobacter calcoaceticus complex	R			[a]	R						R			R				R		R	R
Burkholderia cepacia complex	R	R	R	R	R	R	R	R		R	R	R		R	R	R		R			R
Pseudomonas aeruginosa	R			R	R		R	R						R			R	R	R	R	
Stenotrophomonas maltophilia	R	R	R	R	R	R	R	R			R	R	R	R		R	[b]	R			R

Abbreviation: R, resistant.

NOTE: These nonfermentative gram-negative bacteria are also intrinsically resistant to penicillin (ie, benzylpenicillin), cephalosporins I (cephalothin, cefazolin), cephalosporin II (cefuroxime), cephamycins (cefoxitin, cefotetan), clindamycin, daptomycin, fusidic acid, glycopeptides (vancomycin, teicoplanin), linezolid, macrolides (erythromycin, azithromycin, clarithromycin), quinupristin-dalfopristin, and rifampin.

Footnotes

a. *A. baumannii/calcoaceticus* may appear to be susceptible to ampicillin-sulbactam due to the activity of sulbactam with this species.

b. *S. maltophilia* is intrinsically resistant to tetracycline but not to doxycycline, minocycline, or tigecycline.

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Appendix B. (Continued)

B3. Staphylococci

Organism \ Antimicrobial Agent	Novobiocin	Fosfomycin	Fusidic Acid
S. aureus/S. lugdunensis	There is no intrinsic resistance in these species.		
S. epidermidis			
S. haemolyticus			
S. saprophyticus	R	R	R
S. capitis		R	
S. cohnii	R		
S. xylosus	R		

Abbreviation: R, resistant.

NOTE 1: These gram-positive bacteria are also intrinsically resistant to aztreonam, polymyxin B/colistin, and nalidixic acid.

NOTE 2: Oxacillin-resistant *S. aureus* and coagulase-negative staphylococci (methicillin-resistant staphylococci [MRS]) are considered resistant to other β-lactam agents, ie, penicillins, β-lactam **combination agents**, cephems (with the exception of the cephalosporins with anti-MRSA [methicillin-resistant *S. aureus*] activity), and carbapenems. This is because most cases of documented MRS infections have responded poorly to β-lactam therapy, or because convincing clinical data that document clinical efficacy for those agents have not been presented.

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Appendix B. (Continued)

B4. *Enterococcus* spp.

Organism \ Antimicrobial Agent	Cephalosporins	Vancomycin	Teicoplanin	Aminoglycosides	Clindamycin	Quinupristin-dalfopristin	Trimethoprim	Trimethoprim-sulfamethoxazole	Fusidic Acid
E. faecalis	R*			R*	R*	R	R	R*	R
E. faecium	R*			R*	R*		R	R*	R
E. gallinarum/E. casseliflavus	R*	R		R*	R*	R	R	R*	R

Abbreviation: R, resistant.

* **Warning:** For *Enterococcus* spp., cephalosporins, aminoglycosides (except for high-level resistance testing), clindamycin, and trimethoprim-sulfamethoxazole may appear active *in vitro,* but are not effective clinically and should not be reported as susceptible.

NOTE: These gram-positive bacteria are also intrinsically resistant to aztreonam, polymyxin B/colistin, and nalidixic acid.

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Appendix B. (Continued)

B5. Anaerobic Gram-Positive Bacilli

Organism / Antimicrobial Agent	Vancomycin	Aminoglycosides
Clostridium spp.		R
Clostridium innocuum	R	R

Abbreviation: R, resistant.

B6. Anaerobic Gram-Negative Bacilli

Organism / Antimicrobial Agent	Aminoglycosides	Penicillin	Ampicillin	Quinolones
Bacteroides spp.	R	R	R	
Fusobacterium canifelinum	R			R

Abbreviation: R, resistant.

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Appendix C. QC Strains for Antimicrobial Susceptibility Tests

QC Strains	Organism Characteristics	Disk Diffusion Tests	MIC Tests	Other Tests	Comments
Acinetobacter baumannii **NCTC 13304**[a,b]	• **OXA-27 (carbapenemase)**	• **β-lactam combination agents**	• **β-lactam combination agents**		
Bacteroides fragilis ATCC®[c] 25285	• β-lactamase positive		• All anaerobes		
Bacteroides thetaiotaomicron ATCC® 29741	• β-lactamase positive		• All anaerobes		
Clostridioides **(formerly** *Clostridium***)** ***difficile*** ATCC® 700057	• β-lactamase negative		• Gram-positive anaerobes		
*Eggerthella lenta (*formerly *Eubacterium lentum)* ATCC® 43055			• All anaerobes		• Growth on Brucella medium not optimal • **No longer required when establishing new QC ranges due to organism variability**
Enterococcus faecalis ATCC® 29212			• Nonfastidious gram-positive bacteria	• Vancomycin agar • HLAR **tests** • High-level mupirocin resistance MIC test	• Assess suitability of medium for sulfonamide or trimethoprim MIC and disk diffusion tests.[d] • Assess suitability of cation content in each batch/lot of MHB for daptomycin broth microdilution. Agar dilution has not been validated for daptomycin.
E. faecalis ATCC® 33186					• Alternative to *E. faecalis* ATCC® 29212 to assess suitability of MHA for sulfonamide or trimethoprim disk diffusion tests.[d]
E. faecalis ATCC® 51299	• *vanB* (vancomycin resistant) • Resistant to high-level aminoglycosides			• Vancomycin agar • HLAR **tests**	

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Appendix C. (Continued)

QC Strains	Organism Characteristics	Disk Diffusion Tests	MIC Tests	Other Tests	Comments
Escherichia coli ATCC® 25922	• β-lactamase negative	• Nonfastidious gram-negative bacteria • *Neisseria meningitidis*	• Nonfastidious gram-negative bacteria • *N. meningitidis*		
E. coli ATCC® 35218[a,b,1,2]	• TEM-1	• **β-lactam combination agents**	• **β-lactam combination agents**		
E. coli NCTC 13353[a,b,3]	• CTX-M-15 (ESBL)	• **β-lactam combination agents**	• **β-lactam combination agents**		
Haemophilus influenzae ATCC® 10211					• Assess each batch/lot of HTM for growth capabilities.
H. influenzae ATCC® 49247	• BLNAR	• *H. influenzae* • *Haemophilus parainfluenzae*	• *H. influenzae* • *H. parainfluenzae*		
H. influenzae ATCC® 49766	• Ampicillin susceptible	• *H. influenzae* • *H. parainfluenzae*	• *H. influenzae* • *H. parainfluenzae*		• More reproducible than *H. influenzae* ATCC® 49247 with selected β-lactam agents
Klebsiella pneumoniae ATCC® 700603[a,b]	• SHV-18 (ESBL)[1,2] • **OXA-2** • **Mutations in OMPK35 and OMPK37**	• **β-lactam combination agents**	• **β-lactam combination agents**	ESBL tests	
K. pneumoniae ATCC® BAA-1705™[a,b]	• **KPC-2 (carbapenemase)** • **TEM** • **SHV**	• **β-lactam combination agents**	• **β-lactam combination agents**	• **Carbapenemase tests**	
K. pneumoniae ATCC® BAA-1706™	• Resistant to carbapenems by noncarbapenemase mechanism			• **Carbapenemase tests**	
***K. pneumoniae* ATCC® BAA-2814™[a,b] – previously B21(KP1074)**	• **KPC-3 (carbapenemase)** • **SHV-11** • **TEM-1**	• **β-lactam combination agents**	• **β-lactam combination agents**		• **Higher MIC (see Table 5A-2) and better indicator of antimicrobial agent stability than *K. pneumoniae* BAA-1705™**

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Appendix C. (Continued)

QC Strains	Organism Characteristics	Disk Diffusion Tests	MIC Tests	Other Tests	Comments
Neisseria gonorrhoeae ATCC® 49226	• CMRNG	• *N. gonorrhoeae*	• *N. gonorrhoeae*		
Pseudomonas aeruginosa ATCC® 27853[e]	• Inducible AmpC β-lactamase	• Nonfastidious gram-negative bacteria	• Nonfastidious gram-negative bacteria		• Assess suitability of cation content in each batch/lot of CAMHB.
Staphylococcus aureus ATCC® 25923	• β-lactamase negative • *mecA* negative • ***mupA* negative**	• Nonfastidious gram-positive bacteria		• High-level mupirocin resistance disk diffusion test • Inducible clindamycin resistance disk diffusion test (D-zone test)	• Little value in MIC testing due to its extreme susceptibility to most drugs
S. aureus ATCC® 29213	• Weak β-lactamase–producing strain • *mecA* negative • ***mupA* negative**		• Nonfastidious gram-positive bacteria	• Oxacillin **salt** agar • High-level mupirocin resistance MIC test • Inducible clindamycin resistance MIC test • **Penicillin zone-edge test**	• Assess suitability of cation content in each batch/lot of MHB for daptomycin broth microdilution.
S. aureus ATCC® 43300	• *mecA* positive	• Cefoxitin disk diffusion testing	• Cefoxitin MIC testing	• Oxacillin **salt** agar	
S. aureus ATCC® BAA-976™	• *msr*(A)-mediated macrolide-only resistance			• **Inducible clindamycin resistance MIC test and disk approximation test (D-zone test)**	
S. aureus ATCC® BAA-977™	• Inducible *erm*(A)-mediated macrolide resistance			• **Inducible clindamycin resistance MIC test and disk approximation test (D-zone test)**	

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Appendix C. (Continued)

QC Strains	Organism Characteristics	Disk Diffusion Tests	MIC Tests	Other Tests	Comments
S. aureus ATCC® BAA-1708™	• *mupA*-mediated high-level mupirocin resistance			• High-level mupirocin resistance test	
Streptococcus pneumoniae ATCC® 49619	• Penicillin intermediate by altered penicillin-binding protein	• *S. pneumoniae* • *Streptococcus* spp. • *N. meningitidis*	• *S. pneumoniae* • *Streptococcus* spp. • *N. meningitidis*	• Inducible clindamycin resistance MIC test	

Abbreviations: ATCC®, American Type Culture Collection; BLNAR, β-lactamase negative, ampicillin-resistant; CAMHB, cation-adjusted Mueller-Hinton broth; CMRNG, chromosomally mediated penicillin-resistant *Neisseria gonorrhoeae;* ESBL, extended-spectrum β-lactamase; HLAR, high-level aminoglycoside resistance; HTM, *Haemophilus* test medium; MHA, Mueller-Hinton agar; MHB, Mueller-Hinton broth; MIC, minimal inhibitory concentration; NCTC, National Collection of Type Cultures; QC, quality control.

NOTE: Information in boldface type is new or modified since the previous edition.

Footnotes

a. Careful attention to organism maintenance (eg, minimal subcultures) and storage (eg, −60°C or below) is especially important for these QC strains because spontaneous loss of the plasmid encoding the β-lactamase has been documented. If stored at temperatures above −60°C or if repeatedly subcultured, these strains may lose their resistance characteristics and QC results may be outside the acceptable ranges.

b. To confirm the integrity of the QC strain, test one of the single β-lactam agents highlighted in orange in Tables 4A-2 and 5A-2 by either a disk diffusion or MIC test when the strain is first subcultured from a frozen or lyophilized stock culture. In-range results for the single agent indicate the QC strain is reliable for QC of β-lactam combination agents. It is not necessary to check the QC strain again with a single agent until a new frozen or lyophilized stock culture is put into use.

c. ATCC® is a registered trademark of the American Type Culture Collection. Per ATCC® convention, the trademark symbol is used after "BAA" in each catalog number, in conjunction with the registered ATCC® name.

d. **Disk diffusion and MIC** end points should be easy to read as 80% or greater reduction in growth if **the medium has** acceptable levels of thymidine.

e. **May** develop resistance to β-lactam antimicrobial agents after repeated **subcultures**. Minimize **this risk by subculturing from a frozen or lyophilized stock culture** at least monthly or whenever the strain demonstrates results outside the acceptable range.

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Appendix C. (Continued)

NOTE: Routine QC strains **listed in Tables 2A through 2J** (in "Routine QC Recommendations" boxes at the top of each page) are tested regularly (ie, daily or weekly) to ensure the test system is working and produces results that fall within specified **ranges** listed in M100. The **routine** QC strains recommended in this document should be included if a laboratory performs CLSI reference disk diffusion or MIC testing as described herein. For commercial test systems, manufacturer's recommendations should be followed for all QC procedures. **Other** QC strains are used to assess particular characteristics of a test or test system in select situations or may represent alternative QC strains. For example, *H. influenzae* ATCC® 10211 is more fastidious than *H. influenzae* ATCC® 49247 or *H. influenzae* ATCC® 49766 and is used to ensure HTM can adequately support the growth of patient isolates of *H. influenzae* and *H. parainfluenzae*. QC strains may possess susceptibility or resistance characteristics specific for one or more special tests listed in M02[4] and M07.[5] They can be used to assess a new test, for training new personnel, and for competence assessment, and it is not necessary to include them in routine daily or weekly antimicrobial susceptibility testing QC programs.

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Appendix D. Cumulative Antimicrobial Susceptibility Report for Anaerobic Organisms[1]

NOTE: Isolates collected from selected US hospitals from 1 January 2013 to 31 December 2016.[a]

D1. *Bacteroides fragilis* Group

Anaerobic Organisms	Number of Strains	Ampicillin-sulbactam		Number of Strains	Piperacillin-tazobactam		Number of Strains	Cefoxitin		Number of Strains	Ertapenem		Number of Strains	Imipenem		Number of Strains	Meropenem	
Percent susceptible (%S) and percent resistant (%R)[c]		%S	%R		%S	%R		%S	%R		%S	%R		%S	%R		%S	%R
Breakpoints, µg/mL		≤8/4	≥32/16		≤**16**/4	≥128/4		≤16	≥64		≤4	≥16		≤4	≥16		≤4	≥16
B. fragilis	**129**	**84**	**2**	**1030**	**96**	1	**830**	**100**	**0**	**133**	**82**	**14**	**189**	**97**	1	**1505**	**93**	**5**
B. thetaiotaomicron	**76**	**82**	**5**	**252**	**87**	**0**	**258**	**13**	**54**	**–**	**–**	**–**	**70**	**100**	**0**	**328**	**99**	**0**
B. ovatus	**30**	**80**	**3**	**206**	**94**	**0**	**177**	**20**	**34**	**19[b]**	**84[b]**	**16[b]**	**49**	100	0	**236**	**95**	**1**
B. vulgatus	**20[b]**	**45[b]**	**15[b]**	**168**	92	**0**	**153**	**73**	**14**	—	—	—	**35**	**97**	0	**171**	**96**	**4**
B. uniformis	**19[b]**	**84[b]**	**0[b]**	**78**	**96**	**0**	**72**	**85**	**10**	—	—	—	**19[b]**	100[b]	0[b]	**93**	**100**	0
Parabacteroides distasonis	**27[b]**	**59[b]**	**19[b]**	**92**	**95**	**1**	**82**	**29**	**43**	—	—	—	**26[b]**	**100[b]**	0	**119**	97	2
B. fragilis group without *B. fragilis*	**172**	**74**	**8**	**796**	**91**	**0**	**742**	**36**	**36**	**19[b]**	**84[b]**	**16[b]**	**199**	**100**	0	**947**	**98**	1
B. fragilis group (all 6 species listed)	**301**	**78**	**5**	**1826**	**94**	**0**	**1572**	**70**	**17**	**152**	**82**	**14**	**388**	**98**	**0**	**2052**	**95**	**4**

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Appendix D. (Continued)

D1. *Bacteroides fragilis* Group (Continued)

Anaerobic Organisms	Number of Strains	Clindamycin		Number of Strains	Moxifloxacin		Number of Strains	Metronidazole[b]	
Percent susceptible (%S) and percent resistant (%R)[c]		%S	%R		%S	%R		%S	%R
Breakpoints, µg/mL		≤2	≥8		≤2	≥8		≤8	≥32
B. fragilis	**1013**	**26**	**22**	**256**	**61**	**32**	**1140**	**100**	**0**
B. thetaiotaomicron	**328**	**28**	**49**	**70**	**54**	**36**	**322**	100	0
B. ovatus	**207**	**46**	**51**	**59**	**41**	**25**	**236**	**100**	0
B. vulgatus	**171**	**53**	**46**	**29[b]**	**31[b]**	**45[b]**	**186**	100	0
B. uniformis	**87**	**45**	**48**	**25[b]**	**48[b]**	**40[b]**	**89**	**100**	0
Parabacteroides distasonis	**108**	**43**	**44**	**37**	**62**	**35**	**118**	100	0
B. fragilis group without *B. fragilis*	**901**	**40**	**48**	**220**	**48**	**35**	**951**	100	0
B. fragilis group (all 6 species listed)	**1914**	**33**	**34**	**476**	**55**	**33**	**2091**	**100**	**0**

Footnotes

a. **Data were generated from unique isolates from patient specimens submitted to Tufts Medical Center, Boston, Massachusetts; International Health Management Associates, Inc., Schaumburg, Illinois; R.M. Alden Research Laboratory, Culver City, California; Creighton University School of Medicine, Omaha, Nebraska; Mayo Clinic College of Medicine and Science, Rochester, Minnesota; and the Centers for Disease Control and Prevention, Atlanta, Georgia. All testing was performed by the agar dilution method. Information and analysis of previous versions of this table have been published.**

b. Calculated from fewer than the CLSI document M39[1] recommendation of 30 isolates.

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Appendix D. (Continued)

c. Intermediate category is not shown, but can be derived by subtraction of %S and %R for each antimicrobial agent from %100.

NOTE: Information in boldface type is new or modified since the previous edition.

Reference for D1

1 CLSI. *Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data; Approved Guideline—Fourth Edition.* CLSI document M39-A4. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.

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Appendix D. (Continued)

NOTE: Isolates collected from selected US hospitals from 1 January 2013 to 31 December 2016.[a]

D2. Anaerobic Organisms Other Than *Bacteroides fragilis* Group

Anaerobic Organisms	Number of Strains	Ampicillin-sulbactam		Number of Strains	Piperacillin-tazobactam		Number of Strains	Imipenem		Number of Strains	Meropenem		Number of Strains	Penicillin	
Percent susceptible (%S) and percent resistant (%R)[d]		%S	%R		%S	%R		**%S**	**%R**		%S	%R		**%S**	**%R**
Breakpoints, µg/mL		≤8/4	≥32/16		≤32/4	≥128/4		**≤4**	**≥16**		≤4	≥16		**≤0.5**	**≥2**
Prevotella spp.	**29**[b]	**97**[b]	**3**[b]	**63**	**100**	**0**	**29**[b]	**100**	**0**	**92**	**98**	0	**63**	**100**	**0**
Fusobacterium **spp.**[b]	**20**[b]	100[b]	0[b]	**55**	**96**	**2**	**75**	**95**	**4**	**20**[b]	100[b]	0[b]	—[f]	—[f]	—[f]
Anaerobic gram-positive cocci[e]	—[f]	—[f]	—[f]	**1853**	99	**1**	**134**	**99**	**0**	**1647**	**100**	**0**	**1647**	**100**	**0**
Cutibacterium* (formerly *Propionibacterium*) *acnes	—[f]	—[f]	—[f]	**18**[b]	100[b]	0[b]	**17**[b]	**94**[b]	**0**[b]	—[f]	—[f]	—[f]	—[f]	—[f]	—[f]
Clostridium perfringens	**15**[b]	100[b]	0	**410**	100	0	**23**[b]	**100**[b]	**0**[b]	**417**	100	0	**402**	**90**	**4**
Clostridioides* (formerly *Clostridium*) *difficile[c]	**76**	**99**	0	**542**	93	**0**	**480**	**69**	**4**	**609**	**99**	0	**533**	**6**	**37**
Other *Clostridium* spp.	—[f]	—[f]	—[f]	**439**	**94**	**1**	**71**	**99**	**0**	**390**	**100**	0	**390**	**69**	**13**

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Appendix D. (Continued)

D2. Anaerobic Organisms Other Than *Bacteroides fragilis* Group (Continued)

Anaerobic Organisms	Number of Strains	Clindamycin		Number of Strains	Moxifloxacin		Number of Strains	Metronidazole	
Percent susceptible (%S) and percent resistant (%R)[d]		%S	%R		%S	%R		%S	%R
Breakpoints in µg/mL		≤2	≥8		≤2	≥8		≤8	≥32
Prevotella spp.	**29[b]**	**69[b]**	**28[b]**	**92**	**66**	**25**	**92**	**99**	0
Fusobacterium **spp.**[b]	**75**	**77**	**21**	**75**	**68**	**23**	**75**	**95**	**5**
Anaerobic gram-positive cocci[e]	**1826**	**97**	**3**	**300**	**72**	**21**	**1692**	**100**	**0**
C.* (formerly *P.*) *acnes	**17[b]**	**53[b]**	**35[b]**	**114**	**95**	**4**	**18[b]**	**0[b]**	**100[b]**
C. perfringens	**425**	**83**	**12**	**23[b]**	**83[b]**	**9[b]**	**425**	100	0
Clostridioides* (formerly *Clostridium*) *difficile[c]	**1013**	**32**	**38**	**480**	**74**	**25**	**1343**	100	0
Other *Clostridium* spp.	**461**	**67**	**25**	**71**	**62**	**35**	**461**	**100**	**0**

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Appendix D. (Continued)

Footnotes

a. **Data were generated from unique isolates from patient specimens submitted to Tufts Medical Center, Boston, Massachusetts; International Health Management Associates, Inc., Schaumburg, Illinois; R.M. Alden Research Laboratory, Culver City, California; Creighton University School of Medicine, Omaha, Nebraska; Mayo Clinic College of Medicine and Science, Rochester, Minnesota; and the Centers for Disease Control and Prevention, Atlanta, Georgia. All testing was performed by the agar dilution method. Information and analysis of previous versions of this table have been published.**

b. Calculated from fewer than the CLSI document M39[1] recommendation of 30 isolates.

c. ***Clostridioides*** **(formerly** *Clostridium***)** *difficile* isolates are from an intestinal source; these results do not imply efficacy for intraluminal infections. Vancomycin minimal inhibitory concentrations for isolates were < 4 µg/mL.

d. Intermediate category is not shown but can be derived by subtraction of %S and %R for each antimicrobial agent from %100.

e. Anaerobic gram-positive cocci include *Peptococcus, Peptostreptococcus, Finegoldia, Peptoniphilus,* and *Anaerococcus* species.

f. The dash (–) symbol indicates that data were not available.

NOTE: Information in boldface type is new or modified since the previous edition.

Reference for D2

[1] CLSI. *Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data; Approved Guideline—Fourth Edition.* CLSI document M39-A4. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.

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Appendix E. Dosage Regimens Used to Establish Susceptible or Susceptible-Dose Dependent Breakpoints

The evolving science of pharmacokinetics-pharmacodynamics has become increasingly important in recent years in determining minimal inhibitory concentration (MIC) breakpoints. Recently approved susceptible or susceptible-dose dependent (SDD) breakpoints for a number of agents have been based on a specific dosage regimen(s); these dosage regimens are listed in the table below. Proper application of the breakpoints necessitates drug exposure at the site of infection that corresponds to or exceeds the expected systemic drug exposure at the dose listed in adult patients with normal renal function. This information should be shared with pharmacists, infectious diseases staff, and others making dosing recommendations for the institution.

Antimicrobial Agent	Breakpoints and Interpretive Categories			
	Susceptible		SDD	
	MIC	Dose	MIC	Dose
Table 2A. *Enterobacteriaceae*				
Azithromycin (*Salmonella* Typhi)	**≤16 µg/mL**	**500 mg daily**	**N/A**	
Aztreonam	≤4 µg/mL	1 g every 8 h	N/A	
Cefazolin	≤2 µg/mL	2 g every 8 h	N/A	
Ceftaroline	≤0.5 µg/mL	600 mg every 12 h	N/A	
Cefepime	≤2 µg/mL	1 g every 12 h	4 µg/mL	1 g every 8 h or 2 g every 12 h
			8 µg/mL or zone diameter: 19–24 mm	2 g every 8 h (Because it is not possible to correlate specific zone diameters with specific MICs, an isolate with a zone diameter in the SDD range should be treated as if it might be an MIC of 8 µg/mL.)
Cefotaxime	≤1 µg/mL	1 g every 8 h	N/A	
Ceftriaxone	≤1 µg/mL	1 g every 24 h	N/A	
Cefoxitin	≤8 µg/mL	8 g per day (eg, 2 g every 6 h)	N/A	
Cefuroxime	≤8 µg/mL	1.5 g every 8 h	N/A	
Ceftazidime	≤4 µg/mL	1 g every 8 h	N/A	
Ceftazidime-avibactam	**≤8/4 µg/mL**	**2.5 g (2 g ceftazidime + 0.5 g avibactam) every 8 h over 2 h**	**N/A**	
Ceftizoxime	≤1 µg/mL	1 g every 12 h	N/A	
Ceftolozane-tazobactam	**≤ 2/4 µg/mL**	**1.5 g every 8 h**	**N/A**	
Doripenem	≤1 µg/mL	500 mg every 8 h	N/A	
Ertapenem	≤0.5 µg/mL	1 g every 24 h	N/A	
Imipenem	≤1 µg/mL	500 mg every 6 h or 1 g every 8 h	N/A	
Meropenem	**≤1 µg/mL**	**1 g every 8 h**	**N/A**	

Appendix E. (Continued)

Antimicrobial Agent	Breakpoints and Interpretive Categories			
	Susceptible		SDD	
	MIC	Dose	MIC	Dose
Table 2B-1. *Pseudomonas aeruginosa*				
Aztreonam	≤8 µg/mL	1 g every 6 h or 2 g every 8 h	N/A	
Cefepime	≤8 µg/mL	1 g every 8 h or 2 g every 12 h	N/A	
Ceftazidime	≤8 µg/mL	1 g every 6 h or 2 g every 8 h	N/A	
Ceftazidime-avibactam	**≥8/4 µg/mL**	**2.5 g (2 g ceftazidime + 0.5 g avibactam) every 8 h over 2 h**	**N/A**	
Colistin	**≤2 µg/mL**	**90 mg of CBA every 8 h, following a loading dose of 270 mg of CBA (patients with normal renal function)**	**N/A**	
Doripenem	≤2 µg/mL	500 mg every 8 h	N/A	
Imipenem	≤2 µg/mL	1 g every 8 h or 500 mg every 6 h	N/A	
Meropenem	≤2 µg/mL	1 g every 8 h	N/A	
Piperacillin	≤16 µg/mL	3 g every 6 h	N/A	
Piperacillin-tazobactam	≤16/4 µg/mL	3 g every 6 h	N/A	
Ticarcillin	≤16 µg/mL	3 g every 6 h	N/A	
Ticarcillin-clavulanate	≤16/2 µg/mL	3 g every 6 h	N/A	
Table 2B-2. *Acinetobacter* spp.				
Colistin	**≤2 µg/mL**	**90 mg of CBA every 8 h, following a loading dose of 270 mg of CBA (patients with normal renal function)**	**N/A**	
Doripenem	≤2 µg/mL	500 mg every 8 h	N/A	
Imipenem	≤2 µg/mL	500 mg every 6 h	N/A	
Meropenem	≤2 µg/mL	1 g every 8 h or 500 mg every 6 h	N/A	
Table 2C. *Staphylococcus* spp.				
Ceftaroline	≤1 µg/mL	600 mg every 12 h	N/A	
Dalbavancin	**≤0.25 µg/mL**	**1500 mg (single dose) IV over 30 minutes or 1000 mg (two does) followed one week later by 500 mg IV over 30 minutes (adult patients with creatinine clearance ≥30 mL/minute).**	**N/A**	
Oritavancin	**≤0.12 µg/mL**	**1200 mg single IV dose**	**N/A**	
Tedizolid	**≤0.5 µg/mL**	**200 mg every 24 h**	**N/A**	
Telavancin	**≤0.12 µg/mL**	**10 mg/kg every 24 h**	**N/A**	
Table 2D. *Enterococcus* spp.				
Dalbavancin	**≤0.25 µg/mL**	**1500 mg (single dose) IV over 30 minutes or 1000 mg (two does) followed one week later by 500 mg IV over 30 minutes (adult patients with creatinine clearance ≥30 mL/minute).**	**N/A**	
Oritavancin	**≤0.12 µg/mL**	**1200 mg single IV dose**	**N/A**	
Tedizolid	**≤0.5 µg/mL**	**200 mg every 24 h**	**N/A**	
Telavancin	**≤0.25 µg/mL**	**10 mg/kg every 24 h**	**N/A**	
Table 2E. *Haemophilus influenzae* and *Haemophilus parainfluenzae*				
Ceftaroline	≤0.5 µg/mL	600 mg every 12 h	N/A	

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Appendix E. (Continued)

Antimicrobial Agent	Breakpoints and Interpretive Categories			
	Susceptible		SDD	
	MIC	Dose	MIC	Dose
Table 2G. *Streptococcus pneumoniae*				
Ceftaroline (nonmeningitis)	≤0.5 µg/mL	600 mg every 12 h	N/A	
Penicillin (nonmeningitis)	≤2 µg/mL	2 million units every 4 h (12 million units per day)	N/A	
Penicillin parenteral (meningitis)	≤0.06 µg/mL	3 million units every 4 h	N/A	
Table 2H-1. *Streptococcus* spp. β-Hemolytic Group				
Ceftaroline	≤0.5 µg/mL	600 mg every 12 h	N/A	
Dalbavancin	**≤0.25 µg/mL**	**1500 mg (single dose) IV over 30 minutes or 1000 mg (two doses) followed one week later by 500 mg IV over 30 minutes (adult patients with creatinine clearance ≥30 mL/minute).**	**N/A**	
Oritavancin	**≤0.25 µg/mL**	**1200 mg single IV dose**	**N/A**	
Tedizolid	**≤0.25 µg/mL**	**200 mg every 24 h**	**N/A**	
Telavancin	**≤0.12 µg/mL**	**10 mg/kg every 24 h**	**N/A**	
Table 2H-2. *Streptococcus* spp. Viridans Group				
Dalbavancin	**≤0.25 µg/mL**	**1500 mg (single dose) IV over 30 minutes or 1000 mg (two does) followed one week later by 500 mg IV over 30 minutes (adult patients with creatinine clearance ≥30 mL/minute).**	**N/A**	
Oritavancin	**≤0.25 µg/mL**	**1200 mg single IV dose**	**N/A**	
Tedizolid	**≤0.5 µg/mL**	**200 mg every 24 h**	**N/A**	
Telavancin	**≤0.06 µg/mL**	**10 mg/kg every 24 h**	**N/A**	

Abbreviations: **CBA, colistin base activity; IV, intravenous;** MIC, minimal inhibitory concentration; N/A, not applicable; SDD, susceptible-dose dependent.

NOTE: Information in boldface type is new or modified since the previous edition.

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Appendix F. Cefepime Breakpoint Change for *Enterobacteriaceae* and Introduction of the Susceptible-Dose Dependent Interpretive Category

What changed?

The CLSI Subcommittee on Antimicrobial Susceptibility Testing revised the cefepime breakpoints in 2014 and introduced the susceptible-dose dependent (SDD) category with this breakpoint revision. Below is a summary of the changes.

Previous – 2013

Method	Susceptible	Intermediate	Resistant
MIC	≤ 8 µg/mL	16 µg/mL	≥ 32 µg/mL
Zone diameter (disk diffusion)	≥ 18 mm	15–17 mm	≤ 14 mm

Revised – 2014

Method	Susceptible	SDD	Resistant
MIC	≤ 2 µg/mL	4–8 µg/mL	≥ 16 µg/mL
Zone diameter (disk diffusion)	≥ 25 mm	19–24 mm	≤ 18 mm

Abbreviations: MIC, minimal inhibitory concentration; SDD, susceptible-dose dependent.

Why were the cefepime breakpoints reconsidered?

The issue of new breakpoints for cefepime became apparent for several reasons:

- Previous breakpoints were based on a higher dose of cefepime than is often used.
- Clinical failures were noted for isolates with cefepime MICs of 4 and 8 µg/mL, especially when lower doses of cefepime were used.
- There are limited new drugs in the pipeline that show activity against multidrug-resistant gram-negative bacteria; thus, there is a need to optimize use of drugs currently available. Designing susceptibility reports to correlate better with dosages of the drug used is one way to help accomplish this goal.

What does "susceptible-dose dependent" (SDD) mean?

The "susceptible-dose dependent" category implies that susceptibility of an isolate depends on the dosage regimen that is used in the patient. In order to achieve levels that are likely to be clinically effective against isolates for which the susceptibility testing results (either MICs or disk diffusion) are in the SDD category, it is necessary to use a dosage regimen (ie, higher doses, more frequent doses, or both) that results in higher drug exposure than the dose that was used to establish the susceptible breakpoint. Consideration should be given to the maximum approved dosage regimen, because higher exposure gives the highest probability of adequate coverage of an SDD isolate. The dosage regimens used to set the SDD interpretive criterion are provided in Appendix E. The drug label should be consulted for recommended doses and adjustment for organ function.

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Appendix F. (Continued)

NOTE: The SDD interpretation is a new category for antibacterial susceptibility testing, although it has been previously applied for interpretation of antifungal susceptibility test results (see CLSI document **M60[1]**). The concept of SDD has been included within the intermediate category definition for antimicrobial agents. However, this is often overlooked or not understood by clinicians and microbiologists when an intermediate result is reported. The SDD category may be assigned when doses well above those used to calculate the susceptible breakpoint are approved and used clinically, and where sufficient data to justify the designation exist and have been reviewed. When the intermediate category is used, its definition remains unchanged.

SDD is recommended instead of "intermediate" when reporting cefepime results for *Enterobacteriaceae* isolates because there are multiple approved dosing options for cefepime, and SDD highlights the option of using higher doses to treat infections caused by isolates when the cefepime MIC is 4 or 8 µg/mL or the zone is 19 to 24 mm.

Why is SDD being used now?

- It has become apparent that there is a growing need to refine susceptibility reporting to maximize clinicians' use of available drugs.
- Intermediate too often means "resistant" to clinicians because they do not appreciate the full definition of "intermediate."
- SDD is more specific and it conveys what we know—a higher dose can be considered for isolates with MICs (or zones) that fall in this interpretive category.
- SDD is already well established for use in antifungal susceptibility testing.
- It is anticipated that reporting a cefepime SDD result will encourage clinicians to consider the possibility that cefepime may be an option for treatment.
- Antibiotic stewardship programs, which emphasize dosage regimen and duration of therapy options, are increasing awareness of appropriate use of antibiotics. Personnel from these programs should be able to describe the significance to clinicians of an SDD result for cefepime.

How should this change be implemented?

- Meet with the appropriate practitioners at your institution (members of the antimicrobial stewardship team, infectious diseases staff, pathology group, pharmacy, etc.) to inform them of these changes and agree on a plan to inform your clinicians of this change.
- Talk to the manufacturer of your antimicrobial susceptibility testing (AST) device to determine how to implement the revised breakpoints on your device.
  - **NOTE:** Because the US Food and Drug Administration (FDA) has not revised the cefepime breakpoints and commercial manufacturers must use FDA breakpoints, the manufacturer cannot adopt the new CLSI cefepime breakpoints. However, for most systems, you can manually change the breakpoints and implement following a verification study.
- Work with your laboratory information system staff to report "SDD" or "D" for *Enterobacteriaceae* when the cefepime MIC is 4 or 8 µg/mL. Make certain that SDD will be transmitted to the hospital information system and appropriately displayed on reports viewed by clinicians.
- Distribute user-specific educational materials to laboratory staff and clinicians receiving AST results from your laboratory. Examples of these materials can be found on the CLSI Subcommittee on Antimicrobial Susceptibility Testing webpage at www.clsi.org.

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Appendix F. (Continued)

Additional Questions and Answers:

1. Q: Does CLSI recommend a comment to be reported with the new cefepime breakpoints?

   A: If a laboratory chooses to report a comment explaining the SDD range, CLSI recommends the following: "The interpretive criterion for susceptible is based on a dosage regimen of 1 g every 12 h. The interpretive criterion for susceptible-dose dependent is based on dosage regimens that result in higher cefepime exposure, either higher doses or more frequent doses or both, up to approved maximum dosage regimens."

2. Q: Will all intermediate ranges become SDD?

   A: No, the SDD category will be implemented for drug/organism combinations only when there is sufficient evidence to suggest alternative approved dosage regimens may be appropriate for organisms that have MICs or zone diameters between the susceptible and resistant categories.

3. Q: Will SDD be applied to other antimicrobial agents?

   A: CLSI will examine the SDD category possibility for additional drug/organism combinations where multiple dosing options exist (eg, other extended-spectrum cephalosporins).

4. Q: How do we perform a verification study before implementing the new cefepime breakpoints on our AST device?

   A: Guidelines for performance of such a verification study are available (see CLSI document M52[2]).[3]

5. Q: Does SDD apply to all patients and specimen types (eg, pediatric, geriatric, immunosuppressed)?

   A: Yes, in terms of laboratory reporting. Clinicians must decide how to use an SDD result for a specific patient in consideration of all other clinical and physiological parameters for that patient.

6. Q: Do the new cefepime breakpoints apply to *Pseudomonas aeruginosa* and other gram-negative bacteria also?

   A: No, currently they are only applicable to members of the *Enterobacteriaceae.*

7. Q: Is any special QC needed once the SDD breakpoints are implemented?

   A: No, currently recommended routine QC is sufficient.

8. Q: Will it be necessary to report SDD on proficiency testing survey samples?

   A: Sponsors of proficiency testing surveys are aware of the difficulties encountered by laboratories in implementing newer CLSI breakpoints. It is highly unlikely that there will be a mandate to report SDD in the near future, but it would be best to check with your proficiency testing survey provider.

Appendix F. (Continued)

9. Q: If we can implement the revised cefepime breakpoints but cannot facilitate reporting of SDD, can we report "intermediate" instead of SDD?

   A: A decision related to this question should be made following consultation with your laboratory director, antibiotic stewardship team (if available), infectious diseases practitioners, pharmacists, and infection control practitioners.

10. Q: If we can implement the revised cefepime breakpoints but cannot facilitate reporting of SDD, can we report an MIC or zone diameter without an MIC?

    A: A zone diameter should never be reported without an interpretation because there is a high risk of misinterpretation of this value and this poses patient safety issues. There is a lesser danger of reporting an MIC without an interpretation, but this should not be done without an accompanying qualifying comment. See answer to question 9, above.

11. Q: If we are still doing extended-spectrum β-lactamase (ESBL) testing and implement the new cefepime breakpoints, do we change a susceptible or SDD result to resistant for ESBL-positive isolates?

    A: No. When CLSI changed the other cephem breakpoints in 2010, the recommendation to perform routine ESBL testing was eliminated. When using the new cefepime breakpoints, there is no need to perform routine ESBL testing for patient reporting purposes. However, ESBL testing might be done for infection control or epidemiological purposes.

12. Q: What does the dosing information that is given with breakpoints mean?

    A: The evolving science of pharmacokinetics-pharmacodynamics has become increasingly important in recent years in determining MIC breakpoints. Recently approved susceptible or SDD breakpoints for a number of agents have been based on a specific dosage regimen(s); these dosage regimens are listed in Appendix E. Proper application of the breakpoints necessitates drug exposure at the site of infection that corresponds to or exceeds the expected systemic drug exposure, at the dose listed, in adult patients with normal renal function. This information should be shared with pharmacists, infectious diseases staff, and others making dosing recommendations for the institution.

References for Appendix F

[1] **CLSI. *Performance Standards for Antifungal Susceptibility Testing of Yeasts.* 1st ed. CLSI supplement M60. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.**

[2] **CLSI. *Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems.* 1st ed. CLSI guideline M52. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.**

[3] **Patel J, Sharp S, Novak-Weekley S. Verification of antimicrobial susceptibility testing methods: a practical approach. *Clin Microbiol Newslett.* 2013;35(13):103-109.**

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Appendix G. Epidemiological Cutoff Values

G1 Defining Epidemiological Cut Off Values

G1.1 Definitions

Epidemiological cutoff value (ECV) – the minimal inhibitory concentration (MIC) or zone diameter value that separates microbial populations into those with and without phenotypically detectable resistance (non-wild-type [NWT] or wild-type [WT], respectively). The ECV defines the highest MIC or smallest zone diameter for the WT population of isolates.

Example:

	ECVs	
Interpretive Category	**MIC, µg/mL**	**Zone Diameter, mm**
Wild-type	≤4	≥20
Non-wild-type	≥8	≤19

ECV interpretive categories:

- **Wild-type (WT)** – an ECV interpretive category defined by an ECV that describes isolates with no detectable resistance or reduced susceptibility for the antimicrobial (antifungal) agent being evaluated.
- **Non-wild-type (NWT)** – an ECV interpretive category defined by an ECV that describes isolates with detectable resistance and reduced susceptibility for the antimicrobial (antifungal) agent being evaluated.

G1.2 Epidemiological Cutoff Values vs Clinical Breakpoints

ECVs are based on *in vitro* data only, using MIC or zone diameter distributions. ECVs are not clinical breakpoints, and the clinical relevance of ECVs for a particular patient has not yet been identified or approved by CLSI or any regulatory agency.

By contrast, clinical breakpoints are established using MIC distributions, pharmacokinetic-pharmacodynamic data, and clinical outcome data, when available (as described in CLSI document M23[1]).

"Caution": Zone diameter (disk diffusion) and MIC values for which ECVs are defined are not to be interpreted or reported as susceptible, intermediate, or resistant, but rather as WT or NWT. The ECVs should not be used as clinical breakpoints.

G1.3 Establishing Epidemiological Cutoff Values

ECVs are determined by collecting and merging MIC distribution data obtained by testing bacteria or fungi from a variety of sources and then applying statistical techniques for estimating the MIC at the upper end of the WT distribution. Subsequently, corresponding zone diameter data from disk diffusion testing are examined and a disk diffusion ECV is determined, when appropriate. In order to ensure reliability, ECVs are estimated while accounting for both biological (strain-to-strain) variation and MIC/disk assay variation within and between laboratories. They are based on the assumption that the WT distribution of a particular antimicrobial agent/organism combination does not vary geographically or over time.

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Appendix G. (Continued)

Several conditions must be fulfilled in order to generate reliable ECVs. The most important are:

- An ECV can only be determined within a single species for a single agent because of the genetic diversity between species within a genus.
- All MIC values included in the dataset must have been determined using a standard reference method (eg, the CLSI MIC broth dilution method as described in M07,[2] which is also the method outlined in an international reference standard[3]). Similarly, the standard reference disk diffusion method as described in M02[4] must be used when zone diameter ECVs are defined.
- Data must be sourced from at least three separate laboratories and at least 100 unique isolates must be included in the merged dataset.
- MIC values contributed from an individual laboratory dataset should be "on scale" (ie, the MIC is not below the lowest or above the highest concentration tested), whenever possible. This is particularly important for MICs of the presumptive WT strains. Before merging data from individual laboratories, the MIC distribution from each laboratory must be inspected, and if the lowest concentration tested is also the mode, the data must be excluded.
  - Once acceptable data are merged, there are several methods that can be used to estimate the ECV.
    - Visual inspection is the simplest method and is generally acceptable for MIC distributions when there is clear separation of WT and NWT strains. When there is obvious overlap between WT and NWT strains, visual inspection is too subjective to set a reliable ECV.
    - Statistical methods are preferred because they remove potential observer bias from the estimation. The two most widely referenced statistical methods are those described by Turnidge et al.[5] and by Kronvall.[6]
  - Estimation of ECVs from MIC distributions may be supplemented with molecular tests for known resistance genes. The detection of a resistance gene per se in strains with MICs at or below the ECV does not necessarily contradict the choice of ECV, unless it can be accompanied by evidence that the gene is being expressed.

G1.4 Epidemiological Cutoff Value Use by the Medical Microbiology Laboratory

The need for testing and interpreting drug and organism combinations with an ECV but no clinical breakpoint must be discussed with appropriate clinical specialists (eg, antibiotic stewardship, infectious diseases, and pharmacy). While ECVs do not predict clinical outcome, laboratories may consider noting WT or NWT MIC (or zone diameter) interpretations on laboratory reports. Many physicians may choose not to consider using antimicrobial agents with an NWT interpretation, if other therapeutic options are available. However, it is critical that laboratories refrain from reporting report WT as susceptible, or NWT as resistant, as there are insufficient clinical data to support this practice.

ECVs may be used to signal the emergence of resistance, although this application for ECVs is best suited to public health laboratories and surveillance studies.

References for Appendix G1

1 CLSI. *Development of* In Vitro *Susceptibility Testing Criteria and Quality Control Parameters*. 5th ed. CLSI guideline M23. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

2 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

Appendix G. (Continued)

[3] ISO. *Clinical laboratory testing and in vitro diagnostic test systems – Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices – Part 1: Reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases.* ISO 20776-1. Geneva, Switzerland: International Organization for Standardization; 2006.

[4] CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

[5] Turnidge J, Kahlmeter G, Kronvall G. Statistical characterisation of bacterial wild-type MIC value distributions and the determination of epidemiological cut-off values. *Clin Microbiol Infect.* 2006;12(5):418-425.

[6] Kronvall G. Normalized resistance interpretation as a tool for establishing epidemiological MIC susceptibility breakpoints. *J Clin Microbiol.* 2010;48(12):4445-4452.

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Appendix G. (Continued)

G2 Epidemiological Cutoff Value Tables

"Caution": Zone diameter (disk diffusion) and MIC values for which ECVs are defined are not to be interpreted or reported as susceptible, intermediate, or resistant, but rather as WT or NWT. The ECVs should not be used as clinical breakpoints.

ECVs listed in Tables G1, G2, and G3 are only applicable to the species indicated. Currently, there are insufficient data to support their use with other species.

Table G1. ECVs for *Enterobacteriaceae*

Antimicrobial Agent	Disk Content	Zone Diameter ECV, mm		MIC ECV, µg/mL		Comments
		WT	NWT	WT	NWT	
Azithromycin[1-5]	15 µg	≥16	≤15	≤8	≥16	For use with *Shigella flexneri.* See Table 2A for azithromycin and *Salmonella* spp.
	–	–	–	≤16	≥32	For use with *Shigella sonnei*.
Colistin	–	–	–	≤2	≥4	For use with ***Klebsiella* (formerly *Enterobacter*) *aerogenes***, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumoniae,* and *Raoultella ornithinolytica.* **The only approved method for testing colistin is MIC by broth microdilution. Disk diffusion and gradient diffusion methods should not be used.**

Abbreviations: ECV, epidemiological cutoff value; MIC, minimal inhibitory concentration; NWT, non-wild-type; WT, wild-type.

NOTE: Information in boldface type is new or modified since the previous edition.

References for Table G1

1 Klontz KC, Singh N. Treatment of drug-resistant *Shigella* infections. *Expert Rev Anti Infect Ther.* 2015;13(1):69-80.

2 Baker KS, Dallman TJ, Ashton PM, et al. Intercontinental dissemination of azithromycin-resistant shigellosis through sexual transmission: a cross-sectional study. *Lancet Infect Dis.* 2015;15(8):913-921.

3 Heiman KE, Karlsson M, Grass J, et al.; Centers for Disease Control and Prevention (CDC). Notes from the field: *Shigella* with decreased susceptibility to azithromycin among men who have sex with men - United States, 2002-2013. *MMWR Morb Mortal Wkly Rep.* 2014;63(6):132-133.

4 Valcanis M, Brown JD, Hazelton B, et al. Outbreak of locally acquired azithromycin-resistant *Shigella flexneri* infection in men who have sex with men. *Pathology.* 2015;47(1):87-88.

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Appendix G. (Continued)

5 Hassing RJ, Melles DC, Goessens WH, Rijnders BJ. Case of *Shigella flexneri* infection with treatment failure due to azithromycin resistance in an HIV-positive patient. *Infection.* 2014;42(4):789-790.

Table G2. ECVs for *Neisseria gonorrhoeae*

Antimicrobial Agent	MIC ECV, μg/mL		Comments
	WT	NWT	
Azithromycin[1-3]	≤1	≥2	For use with *N. gonorrhoeae*.

Abbreviations: ECV, epidemiological cutoff value; MIC, minimal inhibitory concentration; NWT, non-wild-type; WT, wild-type.

References for Table G2

1 Chisholm SA, Dave J, Ison CA. High-level azithromycin resistance occurs in *Neisseria gonorrhoeae* as a result of a single point mutation in the 23S rRNA genes. *Antimicrob Agents Chemother.* 2010;54(9):3812-3816.

2 Demczuk W, Martin I, Peterson S, et al. Genomic epidemiology and molecular resistance mechanisms of azithromycin-resistant *Neisseria gonorrhoeae* in Canada from 1997 to 2014. *J Clin Microbiol.* 2016;54(5):1304-1313.

3 Grad YH, Harris SR, Kirkcaldy RD, et al. Genomic epidemiology of gonococcal resistance to extended spectrum cephalosporins, macrolides, and fluoroquinolones in the United States, 2000-2013. *J Infect Dis*. 2016;214(10):1579-1587.

Table G3. ECVs for Specific Anaerobic Species

Antimicrobial Agent	MIC ECV, μg/mL		Comments
	WT	NWT	
Vancomycin	≤2	≥4	**For use with *Cutibacterium* (formerly *Propionibacterium*) *acnes*[1-4] and *Clostridioides* (formerly *Clostridium*) *difficile*.[5-7]**

Abbreviations: ECV, epidemiological cutoff value; MIC, minimal inhibitory concentration; NWT, non-wild-type; WT, wild-type.

References for Table G3

1 Citron DM, Kwok YY, Appleman MD. In vitro activity of oritavancin (LY333328), vancomycin, clindamycin, and metronidazole against *Clostridium perfringens, Propionibacterium acnes,* and anaerobic Gram-positive cocci. *Anaerobe.* 2005;11(1-2):93-95.

2 Goldstein EJ, Citron DM, Merriam CV, Warren YA, Tyrrell KL, Fernandez HT. In vitro activities of the new semisynthetic glycopeptide telavancin (TD-6424), vancomycin, daptomycin, linezolid, and four comparator agents against anaerobic gram-positive species and *Corynebacterium* spp. *Antimicrob Agents Chemother.* 2004;48(6):2149-2152.

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Appendix G. (Continued)

3 Oprica C, Nord CE; ESCMID Study Group on Antimicrobial Resistance in Anaerobic Bacteria. European surveillance study on the antibiotic susceptibility of *Propionibacterium acnes. Clin Microbiol Infect.* 2005;11(3):204-213.

4 Tyrrell KL, Citron DM, Warren YA, Fernandez HT, Merriam CV, Goldstein EJ. In vitro activities of daptomycin, vancomycin, and penicillin against *Clostridium difficile, C. perfringens, Finegoldia magna,* and *Propionibacterium acnes. Antimicrob Agents Chemother.* 2006;50(8):2728-2731.

5 Snydman DR, McDermott LA, Jacobus NV, et al. U.S.-based National Sentinel Surveillance Study for the epidemiology of *Clostridium difficile*-associated diarrheal isolates and their susceptibility to fidaxomicin. *Antimicrob Agents Chemother*. 2015;59(10):6437-6443.

6 Goldstein EJ, Citron DM, Tyrrell KL, Merriam CV. Comparative in vitro activities of SMT19969, a new antimicrobial agent, against *Clostridium difficile* and 350 gram-positive and gram-negative aerobic and anaerobic intestinal flora isolates. *Antimicrob Agents Chemother*. 2013;57(10):4872-4876.

7 Goldstein EJ, Babakhani F, Citron DM. Antimicrobial activities of fidazomicin. *Clin Infect Dis*. 2012;55 Suppl 2:S143-8.

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Glossary I (Part 1). β-Lactams: Class and Subclass Designations and Generic Name

In the late 1990s, several authorities were consulted to construct the glossary. The intention was to include all agents that appeared in M100, along with related agents available for human use. Since that time, agents have been added to the glossary as they were introduced to CLSI, and they do not need to be FDA cleared to be included. It cannot be assumed that the list is exhaustive, and it should be noted that some agents are no longer available for human use.

Antimicrobial Class	Antimicrobial Subclass(es)		Agent(s) Included; Generic Name(s)
Penicillins	Penicillinase-labile penicillins[a]	Penicillin	Penicillin
		Aminopenicillin**s**	Amoxicillin Ampicillin
		Carboxypenicillin**s**	Carbenicillin Ticarcillin
		Ureidopenicillin**s**	Azlocillin Mezlocillin Piperacillin
	Penicillinase-stable penicillins[b]		Cloxacillin Dicloxacillin Methicillin Nafcillin Oxacillin
	Aminopenicillin		Mecillinam
β-lactam **combination agents**			Amoxicillin-clavulanate Ampicillin-sulbactam Aztreonam-avibactam Cefepime-tazobactam **(1:1)** **Cefepime-zidebactam** Ceftaroline-avibactam Ceftazidime-avibactam Ceftolozane-tazobactam Imipenem-relebactam Meropenem-vaborbactam Piperacillin-tazobactam Ticarcillin-clavulanate
Cephems (parenteral)	Cephalosporin**s** I[c]		Cefazolin Cephalothin Cephapirin Cephradine
	Cephalosporin**s** II[c]		Cefamandole Cefonicid Cefuroxime (parenteral)
	Cephalosporin**s** III[c]		Cefoperazone Cefotaxime Ceftazidime Ceftizoxime Ceftriaxone
	Cephalosporin**s** IV[c]		Cefepime Cefpirome

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Glossary I (Part 1). (Continued)

Antimicrobial Class	Antimicrobial Subclass(es)	Agent(s) Included; Generic Name(s)
Cephems (parenteral (Continued)	Cephalosporins with anti-MRSA activity	Ceftaroline Ceftobiprole
	Cephamycin**s**	Cefmetazole Cefotetan Cefoxitin
	Oxacephem	Moxalactam
	Siderophore cephalosporin	**Cefiderocol**
Cephems (oral)	Cephalosporin**s**	Cefaclor Cefadroxil Cefdinir Cefditoren Cefetamet Cefixime Cefpodoxime Cefprozil Ceftibuten Cefuroxime (oral) Cephalexin Cephradine
	Carbacephem	Loracarbef
Monobactams		Aztreonam
Penems	Carbapenem**s**	Biapenem Doripenem Ertapenem Imipenem Meropenem Razupenem
	Penems	Faropenem Sulopenem

Abbreviation**s**: MRSA, methicillin-resistant *Staphylococcus aureus;* **FDA, US Food and Drug Administration**.

Footnotes

a. Hydrolyzed by staphylococcal penicillinase.

b. Not hydrolyzed by staphylococcal penicillinase.

c. Cephalosporins I, II, III, and IV are sometimes referred to as first-, second-, third-, and fourth-generation cephalosporins, respectively. Cephalosporins III and IV are also referred to as "extended-spectrum cephalosporins." This does not imply activity against extended-spectrum β-lactamase–producing gram-negative bacteria.

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Glossary I (Part 2). Non–β-Lactams: Class and Subclass Designations and Generic Name

In the late 1990s, several authorities were consulted to construct the glossary. The intention was to include all agents that appeared in M100, along with related agents available for human use. Since that time, agents have been added to the glossary as they were introduced to CLSI, and they do not need to be FDA cleared to be included. It cannot be assumed that the list is exhaustive, and it should be noted that some agents are no longer available for human use.

Antimicrobial Class	Antimicrobial Subclass(es)	Agent(s) Included; Generic Name(s)
Aminocyclitols		Spectinomycin
Aminoglycosides		Amikacin Gentamicin Kanamycin Netilmicin Plazomicin Streptomycin Tobramycin
Aminoglycoside-fosfomycin		Amikacin-fosfomycin
Ansamycins	**Rifamycins**	**Rifabutin** **Rifapentine** Rifampin Rifaximin
Folate pathway **antagonists**	**Dihydrofolate reductase inhibitors**	Iclaprim Sulfonamides Trimethoprim Trimethoprim-sulfamethoxazole
	Sulfonamides	Sulfamethoxazole Sulfisoxazole
	Combination	Trimethoprim-sulfamethoxazole
Fosfomycins		Fosfomycin
Glycopeptides	Glycopeptide	Vancomycin
	Lipoglycopeptides	Dalbavancin Oritavancin Teicoplanin Telavancin Ramoplanin
Lincosamides		Clindamycin Lincomycin
Lipopeptides		Daptomycin Surotomycin
	Polymyxins	Colistin Polymyxin B
Macrocyclic **lactone**		Fidaxomicin

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Glossary I (Part 2). (Continued)

Antimicrobial Class	Antimicrobial Subclass(es)	Agent(s) Included; Generic Name(s)
Macrolides		Azithromycin Clarithromycin Dirithromycin Erythromycin
	Fluoroketolide	Solithromycin
	Ketolide**s**	Nafithromycin Telithromycin
Nitroheterocyclics	**Nitrofuran**	Nitrofurantoin
	Nitroimidazoles	Metronidazole Secnidazole Tinidazole
	Thiazolides	**Nitazoxanide** **Tizoxanide**
Oxazolidinones		Linezolid Tedizolid
Peptide	Magainin	Pexiganan
Phenicols		Chloramphenicol **Thiamphenicol**
Pleuromutilin**s**		Lefamulin **Retapamulin**
Pseudomonic acid		Mupirocin
Quinolones		Cinoxacin Garenoxacin Nalidixic acid
	Benzoquinolizine	Levonadifloxacin
	Fluoroquinolone**s**	Besifloxacin Ciprofloxacin Clinafloxacin Delafloxacin Enoxacin Finafloxacin Fleroxacin Gatifloxacin Gemifloxacin Grepafloxacin Levofloxacin Lomefloxacin Moxifloxacin Norfloxacin Ofloxacin Pefloxacin Sparfloxacin Trovafloxacin Ulifloxacin (prulifloxacin)

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Glossary I (Part 2). (Continued)

Antimicrobial Class	Antimicrobial Subclass(es)	Agent(s) Included; Generic Name(s)
Quinolonyl oxazolidinone		Cadazolid
Steroid	Fusidane	Fusidic acid
Streptogramins		Quinupristin-dalfopristin
Tetracyclines		Doxycycline Minocycline Tetracycline
	Fluorocycline	Eravacycline
	Glycylcycline	Tigecycline
	Aminomethylcycline	Omadacycline
Triazaacenaphthylene		Gepotidacin

Abbreviation: FDA, US Food and Drug Administration.

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Glossary II. Antimicrobial Agent Abbreviation(s), Route(s) of Administration, and Drug Class

In the late 1990s, several authorities were consulted to construct the glossary. The intention was to include all agents that appeared in M100, along with related agents available for human use. Since that time, agents have been added to the glossary as they were introduced to CLSI, and they do not need to be FDA cleared to be included. It cannot be assumed that the list is exhaustive, and it should be noted that some agents are no longer available for human use.

Antimicrobial Agent	Abbreviation(s)[a]	Route(s) of Administration[b]				Drug Class or Subclass
		PO	IM	IV	Topical	
Amikacin	AN, AK, Ak, AMI, AMK		X	X		Aminoglycoside
Amikacin-fosfomycin	AKF	X[c]				Aminoglycoside-fosfomycin
Amoxicillin	AMX, Amx, AMOX, AC	X				Penicillin
Amoxicillin-clavulanate	AMC, Amc, A/C, AUG, Aug, XL, AML	X				β-lactam **combination agent**
Ampicillin	AM, Am, AMP	X	X	X		Penicillin
Ampicillin-sulbactam	SAM, A/S, AMS, AB			X		β-lactam **combination agent**
Azithromycin	AZM, Azi, AZI, AZ	X		X		Macrolide
Azlocillin	AZ, Az, AZL		X	X		Penicillin
Aztreonam	ATM, AZT, Azt, AT, AZM			X		Monobactam
Aztreonam-avibactam	AZA			X		β-lactam **combination agent**
Besifloxacin	BES				X	Fluoroquinolone
Biapenem	BPM			X		Carbapenem
Cadazolid	CDZ	X				Quinolonyl oxazolidinone
Carbenicillin (indanyl salt) Carbenicillin	CB, Cb, BAR	X 	 X	 X		Penicillin
Cefaclor	CEC, CCL, Cfr, FAC, CF	X				Cephem
Cefadroxil	CFR, FAD	X				Cephem
Cefamandole	MA, CM, Cfm, FAM		X	X		Cephem
Cefazolin	CZ, CFZ, Cfz, FAZ, KZ		X	X		Cephem
Cefdinir	CDR, Cdn, DIN, CD, CFD	X				Cephem
Cefditoren	CDN	X				Cephem
Cefepime	FEP, Cpe, PM, CPM		X	X		Cephem
Cefepime-tazobactam	FPT			X		β-lactam **combination agent**
Cefepime-zidebactam	**FPZ**			**X**		**β-lactam/β-lactam enhancer**
Cefetamet	CAT, FET	X				Cephem
Cefiderocol	**FDC**			**X**		**Siderophore β-lactam**
Cefixime	CFM, FIX, Cfe, IX	X				Cephem
Cefmetazole	CMZ, CMZS, CMT		X	X		Cephem
Cefonicid	CID, Cfc, FON, CPO		X	X		Cephem
Cefoperazone	CFP, Cfp, CPZ, PER, FOP, CP		X	X		Cephem
Cefotaxime	CTX, TAX, Cft, FOT, CT		X	X		Cephem
Cefotetan	CTT, CTN, Ctn, CTE, TANS, CN		X	X		Cephem
Cefoxitin	FOX, CX, Cfx, FX		X	X		Cephem
Cefpirome	CPO, CPR		X	X		Cephem

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Glossary II. (Continued)

Antimicrobial Agent	Abbreviation(s)[a]	Route(s) of Administration[b]				Drug Class or Subclass
		PO	IM	IV	Topical	
Cefpodoxime	CPD, Cpd, POD, PX	X				Cephem
Cefprozil	CPR, CPZ, FP	X				Cephem
Ceftaroline	CPT			X		Cephem
Ceftaroline-avibactam	CPA			X		β-lactam **combination agent**
Ceftazidime	CAZ, Caz, TAZ, TZ		X	X		Cephem
Ceftazidime-avibactam	CZA			X		β-lactam **combination agent**
Ceftibuten	CTB, TIB, CB	X				Cephem
Ceftizoxime	ZOX, CZX, CZ, Cz, CTZ, TIZ		X	X		Cephem
Ceftobiprole	BPR			X		Cephem
Ceftolozane-tazobactam	C/T			X		β-lactam **combination agent**
Ceftriaxone	CRO, CTR, FRX, Cax, AXO, TX		X	X		Cephem
Cefuroxime (oral) Cefuroxime (parenteral)	CXM, CFX, ROX, Crm, FUR, XM	X	 X	 X		Cephem
Cephalexin	CN, LEX, CFL	X				Cephem
Cephalothin	CF, Cf, CR, CL, CEP, CE, KF			X		Cephem
Cephapirin	CP, HAP		X	X		Cephem
Cephradine	RAD, CH	X				Cephem
Chloramphenicol	C, CHL, CL	X		X		Phenicol
Cinoxacin	CIN, Cn	X				Quinolone
Ciprofloxacin	CIP, Cp, CI	X		X		Fluoroquinolone
Clarithromycin	CLR, CLM, CLA, Cla, CH	X				Macrolide
Clinafloxacin	CFN, CLX, LF	X		X		Fluoroquinolone
Clindamycin	CC, CM, CD, Cd, CLI, DA	X	X	X		Lincosamide
Colistin	CL, CS, CT			X		Lipopeptide
Dalbavancin	DAL			X		Glycopeptide
Daptomycin	DAP			X		Lipopeptide
Delafloxacin	DFX	**X**		X		Fluoroquinolone
Dicloxacillin	DX, DIC	X				Penicillin
Dirithromycin	DTM, DT	X				Macrolide
Doripenem	DOR			X		Carbapenem
Doxycycline	DOX, DC, DOXY	X		X		Tetracycline
Eravacycline	ERV	X		X		Fluorocycline
Ertapenem	ETP		X	X		Carbapenem
Erythromycin	E, ERY, EM	X		X		Macrolide
Faropenem	FAR, FARO	X				Penem
Fidaxomicin	FDX	X				Macrocyclic
Finafloxacin	FIN	X		X	X	Fluoroquinolone

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Glossary II. (Continued)

Antimicrobial Agent	Abbreviation(s)[a]	Route(s) of Administration[b]				Drug Class or Subclass
		PO	IM	IV	Topical	
Fleroxacin	FLE, Fle, FLX, FO	X		X		Fluoroquinolone
Fosfomycin	FOS, FF, FO, FM	X				Fosfomycin
Fusidic acid	FA, FC	X		X	X	Steroidal
Garenoxacin	GRN	X		X		Quinolone
Gatifloxacin	GAT	X		X		Fluoroquinolone
Gemifloxacin	GEM	X				Fluoroquinolone
Gentamicin Gentamicin synergy	GM, Gm, CN, GEN GM500, HLG, Gms		X	X		Aminoglycoside
Gepotidacin	GEP	X		X		Triazaacenaphthylene
Grepafloxacin	GRX, Grx, GRE, GP	X				Fluoroquinolone
Iclaprim	ICL			X		Folate pathway **antagonist**
Imipenem	IPM, IMI, Imp, IP			X		Carbapenem
Imipenem-relebactam				X		β-lactam **combination agents**
Kanamycin	K, KAN, HLK, KM		X	X		Aminoglycoside
Lefamulin	LMU	X		X		Pleuromutilin
Levofloxacin	LVX, Lvx, LEV, LEVO, LE	X		X		Fluoroquinolone
Levonadifloxacin	LND			X		Benzoquinolizine
Linezolid	LNZ, LZ, LZD	X		X		Oxazolidinone
Lomefloxacin	LOM, Lmf	X				Fluoroquinolone
Loracarbef	LOR, Lor, LO	X				Cephem
Mecillinam	MEC	X				Penicillin
Meropenem	MEM, Mer, MERO, MRP, MP			X		Carbapenem
Meropenem-vaborbactam	MEV			X		β-lactam **combination agent**
Methicillin	DP, MET, ME, SC		X	X		Penicillin
Metronidazole	MTZ	X		X		Nitroimidazole
Mezlocillin	MZ, Mz, MEZ		X	X		Penicillin
Minocycline	MI, MIN, Min, MN, MNO, MC, MH	X		X		Tetracycline
Moxalactam	MOX		X	X		Cephem
Moxifloxacin	MXF	X		X		Fluoroquinolone
Mupirocin	MUP, MOP, MU				X	Pseudomonic acid
Nafcillin	NF, NAF, Naf		X	X		Penicillin
Nafithromycin	ZWK	X				Ketolide
Nalidixic acid	NA, NAL	X				Quinolone
Netilmicin	NET, Nt, NC		X	X		Aminoglycoside
Nitazoxanide	NIT	X				Thiazolide
Nitrofurantoin	F/M, FD, Fd, FT, NIT, NI, F	X				Nitrofurantoin

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Glossary II. (Continued)

Antimicrobial Agent	Abbreviation(s)[a]	Route(s) of Administration[b]				Drug Class or Subclass
		PO	IM	IV	Topical	
Norfloxacin	NOR, Nxn, NX	X				Fluoroquinolone
Ofloxacin	OFX, OFL, Ofl, OF	X	X	X		Fluoroquinolone
Omadacycline	OMC	X		X		Tetracycline
Oritavancin	ORI			X		Lipoglycopeptide
Oxacillin	OX, Ox, OXS, OXA	X	X	X		Penicillin
Pefloxacin	PEF, PF					Fluoroquinolone
Penicillin	P, PEN, PV	X	X	X		Penicillin
Pexiganan	PEX				X	Peptide
Piperacillin	PIP, PI, PP, Pi		X	X		Penicillin
Piperacillin-tazobactam	TZP, PTZ, P/T, PTc			X		β-lactam **combination agent**
Plazomicin	PLZ			X		Aminoglycoside
Polymyxin B	PB			X		Lipopeptide
Quinupristin-dalfopristin	SYN, Syn, QDA, RP			X		Streptogramin
Razupenem	RZM			X		Carbapenem
Ramoplanin	RAM	X				Lipoglycopeptide
Rifampin	RA, RIF, Rif, RI, RD	X		X		Ansamycin
Rifaximin		X				Ansamycin
Secnidazole	SEC	X				Nitroimidazole
Solithromycin	SOL	X		X	X	Fluoroketolide
Sparfloxacin	SPX, Sfx, SPA, SO	X				Fluoroquinolone
Spectinomycin	SPT, SPE, SC		X	X		Aminocyclitol
Streptomycin Streptomycin synergy	S, STR, StS, SM, ST2000, HLS		X	X		Aminoglycoside
Sulfonamides	G, SSS, S3	X		X		Folate pathway **antagonist** (some PO only)
Sulopenem	SLP, SULO	X		X		Penem
Surotomycin	SUR	X				Lipopeptide
Tedizolid	TZD	X		X		Oxazolidinone
Teicoplanin	TEC, TPN, Tei, TEI, TP, TPL		X	X		Glycopeptide
Telavancin	TLV			X		Lipoglycopeptide
Telithromycin	TEL	X				Ketolide
Tetracycline	TE, Te, TET, TC	X		X		Tetracycline
Ticarcillin	TIC, TC, TI, Ti		X	X		Penicillin
Ticarcillin-clavulanate	TIM, Tim, T/C, TCC, TLc			X		β-lactam **combination agent**
Tigecycline	TGC			X		Glycylcycline
Tinoxanide	TIN	X				Thiazolide
Tinidazole	TNZ	X				Nitroimidazoles
Tobramycin	NN, TM, TO, To, TOB		X	X		Aminoglycoside
Trimethoprim	TMP, T, TR, W	X				Folate pathway **antagonist**

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Glossary II. (Continued)

Antimicrobial Agent	Abbreviation(s)[a]	Route(s) of Administration[b]				Drug Class or Subclass
		PO	IM	IV	Topical	
Trimethoprim-sulfamethoxazole	SXT, SxT, T/S, TS, COT	X		X		Folate pathway **antagonist**
Trospectomycin	TBR		X	X		Aminocyclitol
Trovafloxacin	TVA, Tva, TRV, TV	X		X		Fluoroquinolone
Ulifloxacin (prulifloxacin)	PRU	X				Fluoroquinolone
Vancomycin	VA, Va, VAN	X		X		Glycopeptide

Abbreviations: **FDA, US Food and Drug Administration;** PO, oral; IM, intramuscular; IV, intravenous.

Footnotes

a. Abbreviations assigned to one or more diagnostic products in the United States. If no diagnostic product is available, abbreviation is that of the manufacturer.

b. As available in the United States.

c. Amikacin-fosfomycin is aerosolized and inhaled.

NOTE: Information in boldface type is new or modified since the previous edition.

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M100, 28th ed.

For Use With M02 and M07

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Glossary III. List of Identical Abbreviations Used for More Than One Antimicrobial Agent in US Diagnostic Products

In the late 1990s, several authorities were consulted to construct the glossary. The intention was to include all agents that appeared in M100, along with related agents available for human use. Since that time, agents have been added to the glossary as they were introduced to CLSI, and they do not need to be FDA cleared to be included. It cannot be assumed that the list is exhaustive, and it should be noted that some agents are no longer available for human use.

Abbreviation	Antimicrobial Agents for Which Respective Abbreviation Is Used
AZ	Azithromycin, Azlocillin
AZM	Azithromycin, Aztreonam
CB, Cb	Ceftibuten, Carbenicillin
CD, Cd	Clindamycin, Cefdinir
CF, Cf	Cefaclor, Cephalothin
CFM, Cfm	Cefixime, Cefamandole
CFR, Cfr	Cefaclor, Cefadroxil
CFX, Cfx	Cefoxitin, Cefuroxime
CH	Clarithromycin, Cephradine
CL	Cephalothin, Chloramphenicol
CM	Clindamycin, Cefamandole
CN, Cn	Cephalexin, Cefotetan, Cinoxacin, Gentamicin
CP, Cp	Cephapirin, Cefoperazone, Ciprofloxacin
CPZ	Cefprozil, Cefoperazone
CZ, Cz	Ceftizoxime, Cefazolin
DX	Doxycycline, Dicloxacillin
FO	Fleroxacin, Fosfomycin
NIT	Nitazoxanide, Nitrofurantoin
SC	Spectinomycin, Methicillin
SO	Sparfloxacin, Oxacillin
TC	Tetracycline, Ticarcillin

Abbreviation: FDA, US Food and Drug Administration.

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The Quality Management System Approach

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

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

M100 covers the QSE indicated by an "X." For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section.

Organization	Customer Focus	Facilities and Safety	Personnel	Purchasing and Inventory	Equipment	Process Management	Documents and Records	Information Management	Nonconforming Event Management	Assessments	Continual Improvement
						X EP23 M02 M07 M11 M23 M39 M45 M52 M60					

Path of Workflow

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

M100 covers the medical laboratory path of workflow processes indicated by an "X." For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section.

Preexamination				**Examination**			**Postexamination**	
Examination ordering	Sample collection	Sample transport	Sample receipt and processing	Examination	Results review and follow-up	Interpretation	Results reporting and archiving	Sample management
				EP23 M02 M07 M11	X EP23 M02 M07 M11 M45 M60	X EP23 M02 M07 M11 M45 M60	X M02 M07 M11 M39 M45 M60	

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Related CLSI Reference Materials*

EP23™ **Laboratory Quality Control Based on Risk Management. 1st ed., 2011.** This document provides guidance based on risk management for laboratories to develop quality control plans tailored to the particular combination of measuring system, laboratory setting, and clinical application of the test.

M02 **Performance Standards for Antimicrobial Disk Susceptibility Tests. 13th ed., 2018.** This standard covers the current recommended methods for disk susceptibility testing and criteria for quality control testing.

M07 **Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. 11th ed., 2018.** This standard covers reference methods for determining minimal inhibitory concentrations of aerobic bacteria by broth macrodilution, broth microdilution, and agar dilution.

M11 **Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria. 8th ed., 2012.** This standard provides reference methods for the determination of minimal inhibitory concentrations of anaerobic bacteria by agar dilution and broth microdilution.

M23 **Development of *In Vitro* Susceptibility Testing Criteria and Quality Control Parameters. 5th ed., 2018.** This guideline discusses the necessary and recommended data for selecting appropriate breakpoints and quality control ranges for antimicrobial agents.

M39 **Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data. 4th ed., 2014.** This document describes methods for recording and analysis of antimicrobial susceptibility test data, consisting of cumulative and ongoing summaries of susceptibility patterns of clinically significant microorganisms.

M45 **Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria. 3rd ed., 2016.** This guideline informs clinical, public health, and research laboratories on susceptibility testing of infrequently isolated or fastidious bacteria that are not included in CLSI documents M02, M07, or M100. Antimicrobial agent selection, test interpretation, and quality control are addressed.

M52 **Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems. 1st ed., 2015.** This guideline includes recommendations for verification of commercial US Food and Drug Administration–cleared microbial identification and antimicrobial susceptibility testing systems by clinical laboratory professionals to fulfill regulatory or quality assurance requirements for the use of these systems for diagnostic testing.

M60 **Performance Standards for Antifungal Susceptibility Testing of Yeasts. 1st ed., 2017.** This document includes updated minimal inhibitory concentration, zone diameter, and quality control tables for the Clinical and Laboratory Standards Institute antifungal susceptibility testing documents M27 and M44.

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

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