CLINICAL AND LABORATORY STANDARDS INSTITUTE®

31st 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 disk diffusion (M02[1]) and dilution (M07[2] and M11[3]) test procedures for aerobic and anaerobic bacteria. 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

M100-Ed31 replaces the previous edition of the supplement, M100, 30th ed., published in 2020. The major changes in M100-Ed31 are listed below. 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*."**

Users of M100-Ed31 should note recent and new formatting changes to Tables 2, including:

- Intermediate ranges denoted with a ^ for the applicable antimicrobial agents in the drug groups in Tables 2 are based on the known ability of these agents to concentrate in the urine.

M100 is updated and reviewed annually as new data and new agents become available. Use of outdated documents is strongly discouraged.

Section/Table	Change(s)
General	
CLSI Breakpoint Additions/Revisions Since 2010	**Added:** • Azithromycin – Disk diffusion and minimal inhibitory concentration (MIC) breakpoints for *Shigella* spp. (p. xxiii) – Disk diffusion breakpoints for *Neisseria gonorrheoae* (p. xxvii) • Imipenem-relebactam: – Disk diffusion and MIC breakpoints for Enterobacterales and *Pseudomonas aeruginosa* (p. xxiv) – MIC breakpoints for anaerobes (p. xxviii) • Ceftolozane-tazobactam MIC breakpoints for *Haemophilus influenzae* and *Haemophilus parainfluenzae* (p. xxvi) • Lefamulin disk diffusion and MIC breakpoints for *Staphylococcus* spp. (p. xxv), *H. influenzae* and *H. parainfluenzae* (p. xxvi), and *Streptococcus pneumoniae* (p. xxvii) **Revised:** • Cefazolin separated into parenteral and oral new and revised breakpoints (p. xxiii) • Oxacillin MIC breakpoints for *Staphylococcus* spp. except *Staphylococcus aureus* and *Staphylococcus lugdunensis* (p. xxvi)

Overview of Changes (Continued)

Section/Table	Change(s)
General (Continued)	
CLSI Breakpoint Additions/Revisions Since 2010 (Continued)	**Relocated and reorganized:** • Table relocated and placed at the end of the Overview of Changes section (p. xxiii) • Table reorganized for better clarity regarding new and revised breakpoints
CLSI Epidemiological Cutoff Value Additions/Revisions Since 2015	**Relocated:** • Epidemiological cutoff value (ECV) table relocated to Appendix G (p. 268)
CLSI Archived Resources	**Relocated:** • Table relocated and placed after CLSI Breakpoint Additions/Revisions Since 2010 table at the end of the Overview of Changes section (p. xxviii)
Instructions for Use of Tables	
II. Breakpoint and Interpretive Category Definitions	**Revised:** • Note clarifying use of ^ in intermediate interpretive category definition (p. 5)
IIIA. Reporting Results	**Revised:** • Statement regarding isolates for which there are no CLSI breakpoints (pp. 6–7)
VII. Warning	**Revised:** • Warning statement to refrain from reporting results of certain agents for CSF isolates (p. 9)

Overview of Changes (Continued)

Section/Table	Change(s)
Instructions for Use of Tables (Continued)	
X. Abbreviations and Acronyms	**Added:** • BP (breakpoint) (p. 16) • DD (disk diffusion) (p. 16) ***Deleted:*** • NPBP (no previous breakpoint existed)
Tables 1. 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	
Table 1A. Nonfastidious Organisms	**Added:** • Azithromycin to Group B for Enterobacterales for *Salmonella enterica* ser. Typhi and *Shigella* spp. (p. 20) • Imipenem-relebactam to Group B for Enterobacterales and *P. aeruginosa* (p. 20) • Lefamulin to Group B for *S. aureus* (p. 20) **Revised:** • Warning statement to refrain from reporting results of certain agents for CSF isolates (p. 23) **Reorganized:** • Reorganized and reordered the footnotes to adhere to CLSI style
Table 1B. Fastidious Organisms	**Added:** • Ceftolozane-tazobactam to Group C for *H. influenzae* and *H. parainfluenzae* (p. 27) • Lefamulin to Group B for *S. pneumoniae* (p. 26) and to Group C for *H. influenzae* and *H. parainfluenzae* (p. 27) **Revised:** • Warning statement to refrain from reporting results of certain agents for CSF isolates (p. 28) **Reorganized:** • Reorganized and reordered the footnotes to adhere to CLSI style
Table 1C. Anaerobic Organisms	**Added:** • Imipenem-relebactam to Group A (p. 32)

Overview of Changes (Continued)

Section/Table	Change(s)
Tables 2. Zone Diameter and/or MIC Breakpoints	
Table 2A. Zone Diameter and MIC Breakpoints for Enterobacterales	**Added:** • Positive blood culture broth as an inoculum to the testing conditions box (p. 34) • *S. aureus* ATCC® 25923 (for disk diffusion) and *S. aureus* ATCC® 29213 (for MIC) QC testing for azithromycin with *S. enterica* ser. Typhi and *Shigella* spp. (p. 34) • General comment regarding direct blood culture susceptibility testing of Enterobacterales with select antimicrobial agents (p. 35) • Imipenem-relebactam disk diffusion and MIC breakpoints and associated comments (p. 36) • ^ to intermediate range for doripenem, ertapenem, imipenem, and meropenem (pp. 41–42) • Azithromycin disk diffusion and MIC breakpoints and associated comments for *Shigella* spp. (p. 43) • Explanation of ^ symbol (p. 46) **Revised:** • General comment regarding intermediate^ (p. 35) • Dosage regimen comment for ceftazidime-avibactam and meropenem-vaborbactam (p. 36-37)
Table 2B-1. Zone Diameter and MIC Breakpoints for *Pseudomonas aeruginosa*	**Added:** • Imipenem-relebactam disk diffusion and MIC breakpoints and associated comments (p. 49) • Explanation of ^ symbol (p. 51) **Revised:** • General comment regarding intermediate^ (p. 48) • Dosage regimen comment for ceftazidime-avibactam (p. 49)
Table 2C. Zone Diameter and MIC Breakpoints for *Staphylococcus* spp.	**Added:** • General comment and reference regarding species included in the *S. aureus* complex (p. 64) • Comment that linezolid susceptibility as determined by MIC testing predicts tedizolid susceptibility for *S. aureus* (p. 73) • Lefamulin disk diffusion and MIC breakpoints for *S. aureus* and associated comments (p. 73) **Revised:** • General comment and associated table regarding definitive testing methods to detect methicillin (oxacillin) resistance (p. 65) • Oxacillin MIC breakpoints for *Staphylococcus* spp. except *S. aureus* and *S. lugdunensis* (p. 69)

Overview of Changes (Continued)

Section/Table	Change(s)
Tables 2. (Continued)	
Table 2D. Zone Diameter and MIC Breakpoints for *Enterococcus* spp.	**Added:** • Comment that linezolid susceptibility as determined by MIC predicts tedizolid susceptibility for *Enterococcus faecalis* (p. 79) • Explanation of ^ symbol (p. 79) **Revised:** • General comment regarding intermediate^ interpretive category (p. 76)
Table 2E. *Haemophilus influenzae* and *Haemophilus parainfluenzae*	**Added:** • Ceftolozane-tazobactam MIC breakpoint and associated comments for *H. influenzae* (p. 84) • Lefamulin disk diffusion and MIC breakpoints and associated comment for *H. influenzae* (p. 86)
Table 2F. *Neisseria gonorrhoeae*	**Added:** • Azithromycin disk diffusion breakpoint (p. 90)
Table 2G. *Streptococcus pneumoniae*	**Added:** • Lefamulin disk diffusion and MIC breakpoints and associated comments (p. 96)
Table 2H-1. *Streptococcus* spp. ß-Hemolytic Group	**Added:** • Comment that linezolid susceptibility as determined by MIC predicts tedizolid susceptibility for *Streptococcus agalactiae* and *Streptococcus pyogenes* (p. 101)
Table 2H-2. *Streptococcus* spp. Viridans Group	**Added:** • Comment that linezolid susceptibility as determined by MIC predicts tedizolid susceptibility for *Streptococcus anginosus* group (p. 104)
Table 2J. Anaerobes	**Added:** • Imipenem-relebactam MIC breakpoints and associated comments (p. 111) ***Deleted:*** • Piperacillin breakpoints (relocated to the archived breakpoints table)

Section/Table	Change(s)
Tables 3. Specialized Resistance Testing (NOTE: Tables following 3D were renumbered to accommodate addition of the new Table 3E.)	
Table 3D. Tests for Colistin Resistance for Enterobacterales and *Pseudomonas aeruginosa*	**Added:** • Clarification of colistin formulation to use in the colistin broth disk elution test and colistin agar test (p. 142)
Table 3E. Test for Performing Disk Diffusion Directly From Positive Blood Culture Broth (new table)	**Added:** • New table with instructions for performing the disk diffusion test directly from positive blood culture broth (p. 148)
Tables 3G-1 and 3G-2 (formerly 3F). Tests for Detecting Methicillin (Oxacillin) Resistance in *Staphylococcus* spp.	**Revised to create two separate tables:** • **Table 3G-1.** Test for Detecting Methicillin (Oxacillin) Resistance in *Staphylococcus aureus* and *Staphylococcus lugdunensis* (p. 154) • **Table 3G-2.** Test for Detecting Methicillin (Oxacillin) Resistance in *Staphylococcus* spp. Except *Staphylococcus aureus* and *Staphylococcus lugdunensis* (p. 156)
Tables 4. Disk Diffusion QC Ranges and Associated Tables	
Table 4A-1. Disk Diffusion QC Ranges for Nonfastidious Organisms and Antimicrobial Agents Excluding ß-Lactam Combination Agents	**Revised:** • Amikacin QC ranges for *P. aeruginosa* ATCC® 27853 (p. 170) • Ceftobiprole disk (5 µg) and QC ranges for *Escherichia coli* ATCC® 25922 and *S. aureus* ATCC® 25923 (p. 170) • Eravacycline QC range for *E. coli* ATCC® 25922 (p. 171) ***Deleted*:** • Ceftobiprole disk (30 µg) and QC range for *P. aeruginosa* ATCC® 27853 (relocated to the archived QC ranges table)

Overview of Changes (Continued)

Section/Table	Change(s)
Tables 5. MIC QC Ranges and Associated Tables	
Table 5A-2. MIC QC Ranges for Nonfastidious Organisms and β-Lactam Combination Agents	**Added:** • Aztreonam QC range: – *Klebsiella pneumoniae* ATCC® BAA-2814™ • Aztreonam-nacubactam QC ranges: – *E. coli* ATCC® 25922 – *P. aeruginosa* ATCC® 27853 – *K. pneumoniae* ATCC® 700603 – *K. pneumoniae* ATCC® BAA-2814™ • Cefepime QC range: – *K. pneumoniae* ATCC® BAA-2814™ • Cefepime-nacubactam QC ranges: – *E. coli* ATCC® 25922 – *P. aeruginosa* ATCC® 27853 – *K. pneumoniae* ATCC® 700603 – *K. pneumoniae* ATCC® BAA-2814™
Table 5G. MIC Troubleshooting Guide	**Added:** • Guidance for troubleshooting out-of-range MIC QC for *S. pneumoniae* ATCC® 49619 with various antimicrobial agents (p. 212) **Corrected:** • Observation and probable cause for high QC ranges with aminoglycosides and *P. aeruginosa* ATCC® 27853 (p. 211)

Overview of Changes (Continued)

Section/Table	Change(s)
Tables 6. Preparing Antimicrobial Agent Stock Solutions	
Table 6A. Solvents and Diluents for Preparing Stock Solutions of Antimicrobial Agents	***Deleted***: • Meropenem-vaborbactam
Table 6C. Preparing Solutions and Media Containing Combinations of Antimicrobial Agents	**Added:** • Aztreonam-nacubactam (p. 222) • Cefepime-nacubactam (p. 223)
Appendixes	
Appendix A. Suggestions for Confirming Antimicrobial Susceptibility Test Results and Organism Identification for Agents Approved by the US Food and Drug Administration for Clinical Use	**Added:** • Ceftolozane-tazobactam in Category 1 for *H. influenzae* (p. 234) • Lefamulin in Category 1 for *H. influenzae* (p. 234), *S. aureus* (p. 235), and *S. pneumoniae* (p. 236)
Appendix E. Dosage Regimens Used to Establish Susceptible or Susceptible-Dose Dependent Breakpoints	**Added:** • Azithromycin for *Shigella* spp. (p. 260) and *N. gonorrheoae* (p. 262) • Ceftolozane-tazobactam for *P. aeruginosa* (p. 261) and *H. influenzae* (p. 262) • Imipenem-relebactam for Enterobacterales (p.261), *P. aeruginosa* (p. 261), and anaerobes (p. 263) • Lefamulin for *S. aureus*, *H. influenzae*, and *S. pneumoniae* (p. 262)
Appendix G. Epidemiological Cutoff Values	***Deleted***: • Table G1, ECV for Enterobacterales (now *Shigella* spp.), relocated to the archived ECV table
Appendix H. Using Molecular Assays for Resistance Detection; Table H3. Reporting Results From Extended-Spectrum ß-Lactamase Resistance and Carbapenemase Molecular Tests for Enterobacterales	**Added:** • Guidance for handling discrepancies when performing molecular or phenotypic testing for carbapenemases (p. 285) • Targets for detecting carbapenem resistance in Enterobacterales (pp. 284–287) **Revised:** • Comment regarding isolates producing multiple ß-lactamases (p. 287)

Overview of Changes (Continued)

Section/Table	Change(s)
Appendixes (Continued)	
Appendix I. Cefiderocol Broth Preparation and Reading Broth Microdilution Minimal Inhibitory Concentration End Points; I2. Iron-depleted Cation-adjusted Mueller-Hinton Broth	**Revised:** • Instructions for preparing zinc stock solution and iron-depleted cation-adjusted Mueller-Hinton broth
Glossaries	
Glossary I (Part 1). ß-Lactams: Class and Subclass Designations and Generic Names	**Added:** • Aztreonam-nacubactam • Cefepime-nacubactam
Glossary I (Part 2). Non-ß-Lactams: Class and Subclass Designations and Generic Names	**Revised:** • Antimicrobial class and subclass for exebacase
Glossary II. Antimicrobial Agent Abbreviation(s), Route(s) of Administration, and Drug Class	**Added:** • Column to indicate CLSI-recommended antimicrobial agent abbreviations • Additional antimicrobial agents – Aztreonam-nacubactam – Cefepime-nacubactam – Cloxacillin – Enoxacin – Lincomycin – Methicillin – Novobiocin – Rifamycin – Thiamphenicol

CLSI Breakpoint Additions/Revisions Since 2010

Previous breakpoints can be found in the edition of M100 that precedes the document listed in the column labeled "Date of Addition/Revision (M100 edition)." For example, previous breakpoints for aztreonam are listed in M100-S19 (January 2009).

Antimicrobial Agent	Date of Addition/Revision (M100 edition)	DD BPs		MIC BPs		Comment(s)
		New[a]	Revised[b]	New[a]	Revised[b]	
Enterobacterales						
Azithromycin	January 2015 (M100-S25)	X		X		*S. enterica* ser. Typhi only
	March 2021 (M100-Ed31)	**X**		**X**		***Shigella* spp.** **Previously assigned an ECV**
Aztreonam	January 2010 (M100-S20)		X		X	
Cefazolin (parenteral)	January 2010 (M100-S20)				X	Removed DD BPs January 2010 (M100-S20)
	January 2011 (M100-S21)	X			X	
	January 2016 (M100-S26)	X		X		For uUTIs
Cefazolin (oral)	January 2014 (M100-S24)	X		X		Surrogate test for oral cephalosporins and uUTIs
Cefepime	January 2014 (M100-S24)		X		X	Revised BPs include SDD
Cefiderocol	January 2019 (M100, 29th ed.)			X		
	January 2020 (M100, 30th ed.)	X				
Cefotaxime	January 2010 (M100-S20)		X		X	
Ceftaroline	January 2013 (M100-S23)	X		X		
Ceftazidime	January 2010 (M100-S20)		X		X	
Ceftazidime-avibactam	January 2018 (M100, 28th ed.)	X		X		
Ceftizoxime	January 2010 (M100-S20)		X		X	
Ceftolozane-tazobactam	January 2016 (M100-S26)			X		
	January 2018 (M100, 28th ed.)	X				
Ceftriaxone	January 2010 (M100-S20)		X		X	
Ciprofloxacin	January 2012 (M100-S22)		X		X	
	January 2019 (M100, 29th ed.)		X		X	*Salmonella* spp. (including *S. enterica* ser. Typhi)
Colistin	January 2020 (M100, 30th ed.)			X		Previously assigned an ECV
Doripenem	June 2010 (M100-S20-U)	X		X		
Ertapenem	June 2010 (M100-S20-U)		X		X	
	January 2012 (M100-S22)		X		X	

CLSI Breakpoint Additions/Revisions Since 2010 (Continued)

Antimicrobial Agent	Date of Addition/Revision (M100 edition)	DD BPs		MIC BPs		Comment(s)
		New[a]	Revised[b]	New[a]	Revised[b]	
Enterobacterales (Continued)						
Imipenem	June 2010 (M100-S20-U)		X		X	
Imipenem-relebactam	**March 2021 (M100-Ed31)**	**X**		**X**		
Levofloxacin	January 2013 (M100-S23)		X		X	
	January 2019 (M100, 29th ed.)		X		X	*Salmonella* spp. (including *S. enterica* ser. Typhi)
Meropenem	June 2010 (M100-S20-U)		X		X	
Meropenem-vaborbactam	January 2019 (M100, 29th ed.)	X		X		
Norfloxacin	January 2020 (M100, 30th ed.)	X		X		Reinstated BPs deleted from M100, 29th ed.
Ofloxacin	January 2013 (M100-S23)			X		*Salmonella* spp. (including *S. enterica* ser. Typhi)
Pefloxacin	January 2015 (M100-S25)	X				*Salmonella* spp. (including *S. enterica* ser. Typhi) Surrogate test for ciprofloxacin
Polymyxin B	January 2020 (M100, 30th ed.)			X		
Pseudomonas aeruginosa						
Cefiderocol	January 2019 (M100, 29th ed.)			X		
	January 2020 (M100, 30th ed.)	X				
Ceftazidime-avibactam	January 2018 (M100, 28th ed.)	X		X		
Ciprofloxacin	January 2019 (M100, 29th ed.)		X		X	
Colistin	January 2017 (M100, 27th ed.)				X	
	January 2020 (M100, 30th ed.)				X	
Doripenem	January 2012 (M100-S22)	X		X		
Imipenem	January 2012 (M100-S22)		X		X	
Imipenem-relebactam	**March 2021 (M100-Ed31)**	**X**		**X**		
Levofloxacin	January 2019 (M100, 29th ed.)		X		X	
Meropenem	January 2012 (M100-S22)		X		X	
Norfloxacin	January 2020 (M100, 30th ed.)	X		X		Reinstated BPs deleted from M100, 29th ed.

CLSI Breakpoint Additions/Revisions Since 2010 (Continued)

Antimicrobial Agent	Date of Addition/Revision (M100 edition)	DD BPs		MIC BPs		Comment(s)
		New[a]	Revised[b]	New[a]	Revised[b]	
***Pseudomonas aeruginosa* (Continued)**						
Piperacillin	January 2012 (M100-S22)		X		X	
Piperacillin-tazobactam	January 2012 (M100-S22)		X		X	
Polymyxin B	January 2020 (M100, 30th ed.)				X	
Ticarcillin	January 2012 (M100-S22)		X		X	
Ticarcillin-clavulanate	January 2012 (M100-S22)		X		X	
***Acinetobacter* spp.**						
Cefiderocol	January 2019 (M100, 29th ed.)			X		
	January 2020 (M100, 30th ed.)	X				
Colistin	January 2020 (M100, 30th ed.)				X	
Doripenem	January 2014 (M100-S24)	X		X		
Imipenem	January 2014 (M100-S24)		X		X	
Meropenem	January 2014 (M100-S24)		X		X	
Polymyxin B	January 2020 (M100, 30th ed.)				X	
Stenotrophomonas maltophilia						
Cefiderocol	January 2019 (M100, 29th ed.)			X		
	January 2020 (M100, 30th ed.)	X				
Other Non-Enterobacterales						
Norfloxacin	January 2020 (M100, 30th ed.)	X		X		Reinstated BPs deleted from M100, 29th ed.
***Staphylococcus* spp.**						
Cefoxitin	January 2019 (M100, 29th ed.)		X			*S. epidermidis* Surrogate test for oxacillin
Ceftaroline	January 2013 (M100-S23)	X		X		
	January 2019 (M100, 29th ed.)		X		X	Revised BPs include SDD
Dalbavancin	January 2018 (M100, 28th ed.)			X		
Lefamulin	**March 2021 (M100-Ed31)**	**X**		**X**		
Norfloxacin	January 2020 (M100, 30th ed.)	X		X		Reinstated BPs deleted from M100, 29th ed.

CLSI Breakpoint Additions/Revisions Since 2010 (Continued)

Antimicrobial Agent	Date of Addition/Revision (M100 edition)	DD BPs		MIC BPs		Comment(s)
		New[a]	Revised[b]	New[a]	Revised[b]	
***Staphylococcus* spp. (Continued)**						
Oritavancin	January 2016 (M100-S26)			X		
Oxacillin	January 2016 (M100-S26)		X		X	*S. pseudintermedius*
	January 2018 (M100, 28th ed.)		X		X	*S. schleiferi*
	January 2019 (M100, 29th ed.)		X			*S. epidermidis*
	March 2021 (M100-Ed31)				**X**	***Staphylococcus* spp. except *S. aureus* and *S. lugdunensis***
Tedizolid	January 2016 (M100-S26)			X		
Telavancin	January 2016 (M100-S26)	X		X		
	January 2017 (M100, 27th ed.)					Removed DD BPs January 2017 (M100, 27th ed.)
***Enterococcus* spp.**						
Dalbavancin	January 2018 (M100, 28th ed.)			X		
Daptomycin	January 2019 (M100, 29th ed.)				X	
	January 2020 (M100, 30th ed.)				X	Separated into two sets of BPs: • *Enterococcus* spp other than *Enterococcus faecium* • *E. faecium* (includes SDD)
Norfloxacin	January 2020 (M100, 30th ed.)	X		X		Reinstated BPs deleted from M100, 29th ed.
Oritavancin	January 2016 (M100-S26)			X		
Tedizolid	January 2016 (M100-S26)			X		
Telavancin	January 2016 (M100-S26)	X		X		
	January 2017 (M100, 27th ed.)					Removed DD BPs January 2017 (M100, 27th ed.)
Haemophilus influenzae* and *Haemophilus parainfluenzae						
Ceftaroline	January 2013 (M100-S23)	X		X		
Ceftolozane-tazobactam	**March 2021 (M100-Ed31)**			**X**		
Doripenem	January 2012 (M100-S22)	X		X		
Lefamulin	**March 2021 (M100-Ed31)**	**X**		**X**		

CLSI Breakpoint Additions/Revisions Since 2010 (Continued)

Antimicrobial Agent	Date of Addition/Revision (M100 edition)	DD BPs		MIC BPs		Comment(s)
		New[a]	Revised[b]	New[a]	Revised[b]	
Neisseria gonorrhoeae						
Azithromycin	January 2019 (M100, 29th ed.)			X		Previously assigned as ECV
	March 2021 (M100-Ed31)	**X**				
Streptococcus pneumoniae						
Ceftaroline	January 2013 (M100-S23)	X		X		
Doripenem	January 2012 (M100-S22)			X		
Doxycycline	January 2013 (M100-S23)	X		X		
Lefamulin	**March 2021 (M100-Ed31)**	**X**		**X**		
Tetracycline	January 2013 (M100-S23)		X		X	
***Streptococcus* spp. β-Hemolytic Group**						
Ceftaroline	January 2013 (M100-S23)	X		X		
Dalbavancin	January 2018 (M100, 28th ed.)			X		
Doripenem	January 2012 (M100-S22)			X		
Oritavancin	January 2016 (M100-S26)			X		
Tedizolid	January 2016 (M100-S26)			X		
Telavancin	January 2016 (M100-S26)	X		X		
	January 2017 (M100, 27th ed.)					Removed DD BPs January 2017 (M100, 27th ed.)
***Streptococcus* spp. Viridans Group**						
Ceftolozane-tazobactam	January 2016 (M100-S26)			X		
Dalbavancin	January 2018 (M100, 28th ed.)			X		
Doripenem	January 2012 (M100-S22)			X		
Oritavancin	January 2016 (M100-S26)			X		
Tedizolid	January 2016 (M100-S26)			X		
Telavancin	January 2016 (M100-S26)	X		X		
	January 2017 (M100, 27th ed.)					Removed DD BPs January 2017 (M100, 27th ed.)

CLSI Breakpoint Additions/Revisions Since 2010 (Continued)

Antimicrobial Agent	Date of Addition/Revision (M100 edition)	DD BPs		MIC BPs		Comment(s)
		New[a]	Revised[b]	New[a]	Revised[b]	
Anaerobes						
Doripenem	January 2012 (M100-S22)			X		
Imipenem-relebactam	**March 2021 (M100-Ed31)**			**X**		
Piperacillin-tazobactam	January 2017 (M100, 27th ed.)			X		
	January 2018 (M100, 28th ed.)			X		

Abbreviations: BP, breakpoint; DD, disk diffusion; ECV, epidemiological cutoff value; SDD, susceptible-dose-dependent; uUTI, uncomplicated urinary tract infection.

Footnotes

a. "New" indicates the BPs are listed for the first time for a specific organism or organism group in the respective Table 2.
b. "Revised" indicates previously established BPs for a specific organism or organism group in the respective Table 2 have changed. In some cases, unique BPs were added for a specific genus or species previously included within the organism or organism group BPs (eg, "*Salmonella* spp. [including *S. enterica* ser. Typhi]" was previously grouped with Enterobacterales).

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/pqlom3b5/_m100_archived_drugs_table.pdf
Methods that have been eliminated from M100 have been relocated to the CLSI website.	https://clsi.org/media/nszl4tbc/_m100_archived_methods_table.pdf
QC ranges that have been eliminated from M100 since 2010 have been relocated to the CLSI website.	https://clsi.org/media/r31oari2/_m100_archived_qc_table.pdf
ECVs that have been replaced by breakpoints have been relocated to the CLSI website.	https://clsi.org/media/3mekwxft/_m100_archived_ecvs_table.pdf

Abbreviations: ECV, epidemiological cutoff value; QC, quality control.

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 https://clsi.org/meetings/ast-file-resources/.

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 prevention 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.

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 and interpretive categories 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 are appropriate 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 3K:** tables describing tests to detect particular resistance types in specific organisms or organism groups

I. Selecting Antimicrobial Agents for Testing and Reporting

A. Appropriate Agents for Routine Testing

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 prevention 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 on selected agents may be useful for infection prevention purposes.

B. Equivalent Agents

Antimicrobial agents listed together in a single box are agents for which interpretive categories (susceptible, intermediate, susceptible-dose dependent, 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 (ie, equivalent agents). For example, Enterobacterales 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. **Group A antimicrobial agents,** as listed in Tables 1A, 1B, and 1C, 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 prevention.

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 prevention 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 Enterobacterales in Table 1A, in which cefazolin is listed as a surrogate test 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.

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 prevention 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 Definition

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 on 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 Definition

interpretive category – category derived from microbiological 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 on established breakpoints; **NOTE 2:** See **breakpoint.**

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	>1	<17

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 a zone diameter 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 depends on the dosage regimen that is used in the patient. To achieve levels that are likely to be clinically effective against isolates for which the susceptibility testing results (either MICs or zone diameters) 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 that achieved with the dose that was used to establish the susceptible breakpoint. Consideration should be given to the maximum, literature-supported dosage regimen, because higher exposure gives the highest probability of adequate coverage of an SDD isolate. Appendix E lists the doses used when establishing SDD categories. The drug label should be consulted for recommended doses and adjustment for organ function; **NOTE:** The SDD category may be assigned when doses well above those used to calculate the susceptible breakpoint are supported by the literature, widely used clinically, and/or approved and for which sufficient data to justify the designation exist and have been reviewed. This category also includes a buffer zone for inherent variability in test methods, which should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations, especially for drugs with narrow pharmacotoxicity margins. 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/or for which response rates may be lower than for susceptible isolates; **NOTE:** An I with a ^ in Tables 2 indicates agents that have the potential to concentrate **in the urine**. The I category also includes a buffer zone for inherent variability in test methods, which should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations, especially for drugs with narrow pharmacotoxicity margins.

- **resistant (R)** – a category defined by a breakpoint that implies that isolates with an MIC at or above or a zone diameter 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 the zone diameters are 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 after the time the susceptible-only breakpoint was set; **NOTE 2:** The term “nonsusceptible” should not be used when the text is 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[a]	R	S	I[a]	R
X	30 µg	≥20	15-19	≤14	≤4	8-16	≥32
Y	-	-	-	-	≤1	2	≥4
Z	10 µg	≥16	-	-	≤1	-	-

[a] Or SDD, if appropriate.
Abbreviations: I, intermediate; R, resistant; S, susceptible; SDD, susceptible-dose dependent.

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 or data have not been evaluated as described in CLSI document M23.[4] 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 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, SDD, I, R, or NS) 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 Susceptibility Test Interpretive Criteria (STIC) website (https://www.fda.gov/drugs/development-resources/fda-recognized-antimicrobial-susceptibility-test-interpretive-criteria) and the 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 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 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 prevention personnel, the pharmacy and therapeutics committee, and the antimicrobial stewardship team. See CLSI document M39[6] for detailed instructions on generating cumulative antibiograms.

D. MIC Reporting Concentrations

When serial twofold dilution MICs are being prepared and tested, the actual dilution scheme is, eg:

16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.03125 µg/mL, etc. (see Table 7 for additional dilutions).

For convenience only, not because these are the actual concentrations tested, it was decided to use the following values in **M100**: 16, 8, 4, 2, 1, 0.5, 0.25, 0.12, 0.06, 0.03 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, and laboratories should report an MIC of ≤ 0.125 µg/mL as ≤ 0.12 µg/mL.

IV. Therapy-Related Comments and Dosage Regimens

Some 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 (PK/PD) 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 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 AST 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 Enterobacterales); 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.

VI. Development of Resistance and Testing of Repeat Isolates

Isolates that are initially susceptible may become intermediate or resistant after therapy is initiated. Therefore, subsequent isolates of the same species from a similar anatomical site should be tested 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* (including *Klebsiella* [formerly *Enterobacter*] *aerogenes*), *Citrobacter*, and *Serratia* spp. with third-generation cephalosporins, in *P. aeruginosa* with all antimicrobial agents, and in staphylococci with fluoroquinolones. 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* complex from a blood culture on a premature infant or methicillin (oxacillin)-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.	First- and second-generation cephalosporins, cephamycins, and aminoglycosides
Table 2D	*Enterococcus* spp.	Aminoglycosides (except for high-level resistance testing), cephalosporins, clindamycin, and trimethoprim-sulfamethoxazole
"Warning": Do not report the following antimicrobial agents for bacteria isolated from CSF. These are not the drugs of choice and may not be effective for treating CSF infections caused by 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, **doripenem, ertapenem, imipenem, and lefamulin,** clindamycin, macrolides, tetracyclines, and fluoroquinolones

Abbreviation: CSF, cerebrospinal fluid.

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.

Supplemental Tests (Required)

Supplemental Test	Organisms	Test Description	Required for:	Table Location
Inducible clindamycin resistance	• *Staphylococcus* spp. • *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	**3I**
β-lactamase	• *Staphylococcus* spp.	Chromogenic cephalosporin (all staphylococci), penicillin disk diffusion zone-edge test (*S. aureus* only)	Isolates that test penicillin susceptible before reporting the isolate as penicillin susceptible	**3F**

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	• Enterobacterales • *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: Enterobacterales and *P. aeruginosa* • mCIM with eCIM: Enterobacterales only	Disk diffusion for detecting carbapenem hydrolysis (inactivation) eCIM add-on enables differentiation of metallo-ß-lactamases from serine carbapenemases in Enterobacterales isolates that are positive for mCIM	Isolates demonstrating reduced susceptibility to carbapenems Results that indicate presence or absence of certain carbapenemases	3C
Colistin agar test	• Enterobacterales • *P. aeruginosa*	Modified agar dilution	Determining the colistin MIC	3D
Colistin broth disk elution	• Enterobacterales • *P. aeruginosa*	Tube dilution using colistin disks as antimicrobial agent source	Determining the colistin MIC	3D
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	**3G-1**

Abbreviations: eCIM, EDTA-modified carbapenem inactivation method; ESBL, extended-spectrum ß-lactamase; mCIM, modified carbapenem inactivation method; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; MRSA, methicillin (oxacillin)-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	**3H**
HLAR by disk diffusion	• *Enterococcus* spp.	Disk diffusion with gentamicin and streptomycin	If screen inconclusive	Broth microdilution, agar dilution MIC	**3K**

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

Surrogate Agent Tests

Surrogate Agent	Organisms	Test Description	Results	Table Location
Cefazolin	• *E. coli* • *K. pneumoniae* • *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 **tested** 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
Cefoxitin	• *S. aureus* • *S. lugdunensis* • *S. epidermidis* • Other *Staphylococcus* spp. (except *S. pseudintermedius* and *S. schleiferi*)	Broth microdilution: *S. aureus* *S. lugdunensis* Disk diffusion: *S. aureus* *S. lugdunensis* Other *Staphylococcus* spp., excluding *S. pseudintermedius* and *S. schleiferi*	Predicts results for *mecA*-mediated methicillin (oxacillin) resistance.	1A, 2C, **3G-1, 3G-2**
Oxacillin	• *S. pneumoniae*	Disk diffusion	Predicts penicillin susceptibility if oxacillin zone is ≥ 20 mm. If oxacillin zone is ≤ 19 mm, penicillin MIC must be performed.	1B, 2G
Pefloxacin	• *Salmonella* spp.	Disk diffusion	Predicts reduced susceptibility to ciprofloxacin	2A

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

Examples of Equivalent Agent Tests

Agents	Organisms	Identified by	Table Location
Cefotaxime or ceftriaxone	Enterobacterales	"Or"	1A and 2A
Colistin or polymyxin B	Enterobacterales, *P. aeruginosa*, *Acinetobacter baumannii* complex	"Or"	2A, 2B-1, and 2B-2
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 antimicrobial susceptibility testing (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]).

X. Abbreviations and Acronyms

AST	antimicrobial susceptibility testing
ATCC®[a]	American Type Culture Collection
BHI	brain heart infusion
BLNAR	β-lactamase negative, ampicillin-resistant
BMHA	blood Mueller-Hinton agar
BP	**breakpoint**
BSC	biological safety cabinet
BSL-2	biosafety level 2
BSL-3	biosafety level 3
CAMHB	cation-adjusted Mueller-Hinton broth
CAT	colistin agar test
CBDE	colistin broth disk elution
CFU	colony-forming unit(s)
CMRNG	chromosomally mediated penicillin-resistant *Neisseria gonorrhoeae*
CSF	cerebrospinal fluid
DD	**disk diffusion**
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
ICR	inducible clindamycin resistance
IM	intramuscular
ID	identification
LHB	lysed horse blood

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

mCIM	modified carbapenem inactivation method
MHA	Mueller-Hinton agar
MH-F agar	Mueller-Hinton fastidious agar
MHB	Mueller-Hinton broth
MIC	minimal inhibitory concentration
MRS	methicillin (oxacillin)-resistant staphylococci
MRSA	methicillin (oxacillin)-resistant *Staphylococcus aureus*
NAD	ß-nicotinamide adenine dinucleotide
NCTC	National Collection of Type Cultures
NS	nonsusceptible
NWT	non-wild-type
PBP2a	penicillin-binding protein 2a
PCR	polymerase chain reaction
PK/PD	pharmacokinetic/pharmacodynamic
pH	negative logarithm of hydrogen ion concentration
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

References

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

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

3 CLSI. *Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria*. 9th ed. CLSI standard M11. Clinical and Laboratory Standards Institute; 2018.

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

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

6 CLSI. *Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data; Approved Guideline—Fourth Edition*. CLSI document M39-A4. Clinical and Laboratory Standards Institute; 2014.

7 Centers for Medicare & Medicaid Services, US Department of Health and Human Services. *Part 493—Laboratory Requirements; Standard: Establishment and verification of performance specifications* (Codified at 42 CFR §493.1253). Office of the Federal Register; published annually.

8 CLSI. *Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems*. 1st ed. CLSI guideline M52. Clinical and Laboratory Standards Institute; 2015.

9 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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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

Group A: Includes antimicrobial agents considered appropriate for inclusion in a routine, primary testing panel, as well as for routine reporting of results for the specific organism group.			
Enterobacterales	***Pseudomonas aeruginosa***	***Staphylococcus* spp.**	***Enterococcus* spp.**[a]
Ampicillin[b]	Ceftazidime	Azithromycin[c] or clarithromycin[c] or erythromycin[c]	Ampicillin[d] Penicillin[e]
Cefazolin[f]	Gentamicin Tobramycin		
Gentamicin[b] Tobramycin[b]	Piperacillin-tazobactam	Clindamycin[c]	
		Oxacillin[g,h,i,j,k] Cefoxitin[g,h,j] (surrogate test for oxacillin)	
		Penicillin[g]	
		Trimethoprim-sulfamethoxazole	
Group B: Includes antimicrobial agents that may warrant primary testing but may be reported only selectively, such as when the organism is resistant to agents of the same antimicrobial class in Group A.[l]			
Amikacin[b]	Amikacin	Ceftaroline[m]	Daptomycin[i,n]
Amoxicillin-clavulanate Ampicillin-sulbactam	Aztreonam	Daptomycin[i,n]	Linezolid Tedizolid[o]
Azithromycin[p]			
Ceftazidime-avibactam	Cefepime	Linezolid Tedizolid[m]	Vancomycin
Ceftolozane-tazobactam	Ceftazidime-avibactam		
Imipenem-relebactam	**Imipenem-relebactam**		
Meropenem-vaborbactam	Ceftolozane-tazobactam		
Piperacillin-tazobactam			
Cefuroxime	Ciprofloxacin Levofloxacin	Doxycycline Minocycline[c] Tetracycline[q]	
Cefepime	Doripenem Imipenem Meropenem	**Lefamulin**[m]	
Cefotetan Cefoxitin		Vancomycin[i]	
Cefotaxime[b,f] or Ceftriaxone[b,f]			
Ciprofloxacin[b] Levofloxacin[b]		Rifampin[r]	
Doripenem Ertapenem Imipenem Meropenem			
Trimethoprim-sulfamethoxazole[b]			

Table 1A. (Continued)

Enterobacterales	*Pseudomonas aeruginosa*	*Staphylococcus* spp.	*Enterococcus* spp.[a]
Group C: Includes alternative or supplemental antimicrobial agents that may require testing in institutions that harbor endemic or epidemic strains resistant to several of the primary drugs, for treatment of patients allergic to primary drugs, for treatment of unusual organisms, or for reporting to infection prevention as an epidemiological aid.			
Aztreonam Ceftazidime		Chloramphenicol[c]	Gentamicin (high-level resistance testing only)
Ceftaroline		Ciprofloxacin or levofloxacin Moxifloxacin	Streptomycin (high-level resistance testing only)
Chloramphenicol[b,c]			
Tetracycline[q]			Dalbavancin[i,s]
		Gentamicin[t]	Oritavancin[i,s]
		Dalbavancin[i,m]	Telavancin[i,s]
		Oritavancin[i,m]	
		Telavancin[i,m]	
Group U: Includes antimicrobial agents that are used only or primarily for treating UTIs.			
Cefazolin (surrogate test for uncomplicated UTI)[u]		Nitrofurantoin	Ciprofloxacin Levofloxacin
Fosfomycin[v]		Sulfisoxazole	
Nitrofurantoin		Trimethoprim	Fosfomycin[v]
Sulfisoxazole			Nitrofurantoin
Trimethoprim			Tetracycline[q]

Table 1A. (Continued)

Acinetobacter spp.	*Burkholderia cepacia* complex	*Stenotrophomonas maltophilia*	Other Non-Enterobacterales[i,w]
Group A: Includes antimicrobial agents considered appropriate for inclusion in a routine, primary testing panel, as well as for routine reporting of results for the specific organism group.			
Ampicillin-sulbactam	Levofloxacin[i]	Levofloxacin Minocycline Trimethoprim-sulfamethoxazole	Ceftazidime
Ceftazidime	Meropenem		Gentamicin Tobramycin
Ciprofloxacin Levofloxacin	Trimethoprim-sulfamethoxazole		
Doripenem Imipenem Meropenem			
Gentamicin Tobramycin			
Group B: Includes antimicrobial agents that may warrant primary testing but may be reported only selectively, such as when the organism is resistant to agents of the same antimicrobial class in Group A.[l]			
Amikacin	Ceftazidime	Ceftazidime[i]	Amikacin
Piperacillin-tazobactam	Minocycline		Aztreonam
Cefepime			Cefepime
Cefotaxime Ceftriaxone			Ciprofloxacin Levofloxacin
Doxycycline			Imipenem Meropenem
Minocycline			Piperacillin-tazobactam
Trimethoprim-sulfamethoxazole			Trimethoprim-sulfamethoxazole
Group C: Includes alternative or supplemental antimicrobial agents that may require testing in institutions that harbor endemic or epidemic strains resistant to several of the primary drugs, for treatment of patients allergic to primary drugs, for treatment of unusual organisms, or for reporting to infection prevention as an epidemiological aid.			
	Chloramphenicol[c,i]	Chloramphenicol[c,i]	Cefotaxime Ceftriaxone
			Chloramphenicol[c]
Group U: Includes antimicrobial agents that are used only or primarily for treating UTIs.			
Tetracycline[q]			Sulfisoxazole
			Tetracycline[q]

Abbreviations: CSF, cerebrospinal fluid; MIC, minimal inhibitory concentration; UTI, urinary tract infection.

Table 1A. (Continued)

"Warning": Do not report the following antimicrobial agents for bacteria isolated from CSF. These are not the drugs of choice and may not be effective for treating CSF infections caused by the bacteria included in Tables 2A through 2J:

- Agents administered by oral route only
- First- and second-generation cephalosporins and cephamycins
- **Doripenem, ertapenem, and imipenem**
- Clindamycin
- **Lefamulin**
- Macrolides
- Tetracyclines
- Fluoroquinolones

Refer to Glossary I for individual agents within the drug classes listed above.

Footnotes

a. **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.

b. **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.

Routine susceptibility testing is not indicated for nontyphoidal *Salmonella* spp. isolated from intestinal sources. In contrast, susceptibility testing is indicated for all *Shigella* isolates.

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 if requested, chloramphenicol **and azithromycin** may be tested and reported. Susceptibility testing is indicated for typhoidal *Salmonella* (*S. enterica* ser. Typhi and *Salmonella enterica* ser. Paratyphi A-C) isolated from extraintestinal and intestinal sources.

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

d. 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*.

Table 1A. (Continued)

e. Enterococci susceptible to penicillin are predictably susceptible to ampicillin, amoxicillin, ampicillin-sulbactam, amoxicillin-clavulanate, and piperacillin-tazobactam for non-ß-lactamase-producing enterococci. However, enterococci susceptible to ampicillin cannot be assumed to be susceptible to penicillin. If penicillin results are needed, penicillin testing 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 *Enterococcus*. For strains with low-level penicillin or ampicillin resistance when combination therapy with a ß-lactam is being considered, see additional testing and reporting information in Table **3K.**[1]

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

g. Penicillin-susceptible staphylococci are also susceptible to other ß-lactam agents with established clinical efficacy for staphylococcal infections. Penicillin-resistant staphylococci are resistant to penicillinase-labile penicillins. Methicillin (oxacillin)-resistant staphylococci are resistant to all currently available ß-lactam antimicrobial agents, with the exception of ceftaroline. Thus, susceptibility or resistance to a wide array of ß-lactam antimicrobial agents may be deduced from testing only penicillin and either cefoxitin or oxacillin. Routine testing of other ß-lactam agents, except ceftaroline, is not advised.

h. 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 methicillin (oxacillin) resistance in staphylococci is achieved by using specific methods as described in Tables 2C, **3G-1**, and **3G-2**.

i. MIC testing only; disk diffusion test is unreliable.

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

k. For *S. aureus*, *S. lugdunensis*, and other *Staphylococcus* spp. (except *S. epidermidis*, *S. pseudintermedius*, and *S. schleiferi*), only MIC testing, not disk diffusion testing, is acceptable; see exceptions in Table 2C.

l. Section I, C.2. in the Instructions for Use of Tables lists additional examples of when a Group B agent might be reported.

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

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

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

Table 1A. (Continued)

p. **For reporting against *Salmonella enterica* ser. Typhi and *Shigella* spp. only.**

q. 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.

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

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

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

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

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

w. Other non-Enterobacterales include *Pseudomonas* spp. and other nonfastidious, glucose-nonfermenting, gram-negative bacilli but exclude *P. aeruginosa*, *Acinetobacter* spp., *B. cepacia* complex, and *S. maltophilia*. Refer to each respective organism column for suggested antimicrobial agents to test and report. Recommendations for testing and reporting *Aeromonas* spp., *Burkholderia mallei*, *Burkholderia pseudomallei*, and *Vibrio* spp. (including *V. cholerae*) are found in CLSI document M45.[2]

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.

References for Table 1A

[1] Murray BE, Arias CA, Nannini EC. Glycopeptides (vancomycin and teicoplanin) and lipoglycopeptides (telavancin, oritavancin, and dalbavancin). In: Bennett JE, Dolin R, Blaser MJ. *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*. 9th ed. Philadelphia, PA: Elsevier; 2019: Chapter 30.

[2] CLSI. *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria*. 3rd ed. CLSI guideline M45. Clinical and Laboratory Standards Institute; 2016.

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[a] and *Haemophilus parainfluenzae*	*Neisseria gonorrhoeae*[b]	*Streptococcus pneumoniae*[c]	*Streptococcus* spp. β-Hemolytic Group[d]	*Streptococcus* spp. Viridans Group[d]
Group A: Includes antimicrobial agents considered appropriate for inclusion in a routine, primary testing panel, as well as for routine reporting of results for the specific organism group.				
Ampicillin[a,e]	Azithromycin[f,g]	Erythromycin[h,i]	Clindamycin[i,j]	Ampicillin[f,k] Penicillin[f,k]
	Ceftriaxone[g] Cefixime[g]			
	Ciprofloxacin[g]	Penicillin[l] (oxacillin disk)	Erythromycin[h,i,j]	
	Tetracycline[g]		Penicillin[g,m] or ampicillin[g,m]	
		Trimethoprim-sulfamethoxazole		
Group B: Includes antimicrobial agents that may warrant primary testing but may be reported only selectively, such as when the organism is resistant to agents of the same antimicrobial class, as in Group A.[n]				
Ampicillin-sulbactam		Cefepime[f] Cefotaxime[f,l] Ceftriaxone[f,l]	Cefepime or cefotaxime or ceftriaxone	Cefepime Cefotaxime Ceftriaxone
Cefotaxime[a] or ceftazidime[a] or ceftriaxone[a]		Clindamycin[i]	Vancomycin	Vancomycin
Ciprofloxacin or levofloxacin or moxifloxacin		Doxycycline		
		Lefamulin		
		Levofloxacin[c] Moxifloxacin[c]		
Meropenem[a]		Meropenem[f,l]		
		Tetracycline[o]		
		Vancomycin[l]		

Table 1B. (Continued)

Group C: Includes alternative or supplemental antimicrobial agents that may require testing in institutions that harbor endemic or epidemic strains resistant to several of the primary drugs, for treatment of patients allergic to primary drugs, for treatment of unusual organisms, or for reporting to infection prevention as an epidemiological aid.				
Haemophilus influenzae*[a] and *Haemophilus parainfluenzae	***Neisseria gonorrhoeae*[b]**	***Streptococcus pneumoniae*[c]**	***Streptococcus* spp. β-Hemolytic Group[d]**	***Streptococcus* spp. Viridans Group[d]**
Azithromycin[p] Clarithromycin[p]		Amoxicillin[f] Amoxicillin-clavulanate[f]	Ceftaroline	Ceftolozane-tazobactam
Aztreonam		Cefuroxime[f]	Chloramphenicol[i]	Chloramphenicol[i]
Amoxicillin-clavulanate[p]		Ceftaroline	Daptomycin[f,q]	Clindamycin[i]
Cefaclor[p] Cefprozil[p]		Chloramphenicol[i]	Levofloxacin	Erythromycin[h,i]
Cefdinir[p] or cefixime[p] or cefpodoxime[p]		Ertapenem[f] Imipenem[f]	Linezolid Tedizolid[r]	Linezolid Tedizolid[s]
		Linezolid	Dalbavancin[f,t] Oritavancin[f] Telavancin[f]	Dalbavancin[f,t] Oritavancin[f] Telavancin[f]
Ceftaroline[u]		Rifampin[v]		
Cefuroxime[p]				
Chloramphenicol[i]				
Ceftolozane-tazobactam[u]				
Ertapenem or imipenem				
Lefamulin[u]				
Rifampin[w]				
Tetracycline[o]				
Trimethoprim-sulfamethoxazole				

Abbreviations: CSF, cerebrospinal fluid; MIC, minimal inhibitory concentration.

Table 1B. (Continued)

"Warning": Do not report the following antimicrobial agents for bacteria isolated from CSF. These are not the drugs of choice and may not be effective for treating CSF infections caused by the bacteria included in Tables 2A through 2J:

- Agents administered by oral route only
- First- and second-generation cephalosporins and cephamycins
- **Doripenem, ertapenem, and imipenem**
- Clindamycin
- **Lefamulin**
- Macrolides
- Tetracyclines
- Fluoroquinolones

Refer to Glossary I for individual agents within the drug classes listed above.

Footnotes

a. 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.

b. 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.

c. *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.

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

e. 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.

f. MIC testing only; disk diffusion test is unreliable.

Table 1B. (Continued)

g. Routine testing is not necessary.

h. Susceptibility and resistance to azithromycin, clarithromycin, and dirithromycin can be predicted by testing erythromycin.

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

j. ***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 [ICR]) should be tested, and only clindamycin should be reported. Erythromycin, even when tested for determination of ICR, should not be reported. See Table **3I.**

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

l. 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.

m. 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 *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).

n. Section I, C.2. in the Instructions for Use of Tables lists additional examples of when a Group B agent might be reported.

Table 1B. (Continued)

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

p. 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.

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

r. For reporting against *S. pyogenes* and *S. 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.

u. For reporting against *H. influenzae* only.

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

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

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.

Reference for Table 1B

1 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. 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

Group A: Includes antimicrobial agents considered to be appropriate for inclusion in a routine, primary testing panel, as well as for routine reporting of results for the specific organism group.	
Gram-Negative Anaerobes	**Gram-Positive Anaerobes[a]**
Amoxicillin-clavulanate Ampicillin-sulbactam Piperacillin-tazobactam	Ampicillin[b] Penicillin[b]
	Amoxicillin-clavulanate Ampicillin-sulbactam Piperacillin-tazobactam
Clindamycin	Clindamycin
Doripenem Ertapenem Imipenem **Imipenem-relebactam** Meropenem	Doripenem Ertapenem Imipenem **Imipenem-relebactam** Meropenem
Metronidazole	Metronidazole
Group C: Includes alternative or supplemental antimicrobial agents that may require testing in institutions that harbor endemic or epidemic strains resistant to several of the primary drugs, for treatment of patients allergic to primary drugs, for treatment of unusual organisms, or for reporting to infection prevention as an epidemiological aid.	
Gram-Negative Anaerobes	**Gram-Positive Anaerobes[a]**
Penicillin[b] Ampicillin[b]	Cefotetan Cefoxitin
Cefotetan Cefoxitin	
Ceftizoxime Ceftriaxone	Ceftizoxime Ceftriaxone
Chloramphenicol	
Moxifloxacin	Moxifloxacin
	Tetracycline

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 ß-lactamase-negative isolates may be resistant to penicillin and ampicillin by other mechanisms.

Table 1C. (Continued)

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, *Bacteroides* spp. and *Parabacteroides* spp.) 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 have been reported in *Clostridium perfringens*. Agents in group A of Table 1C should be tested and reported for *Clostridium* spp.

NOTE 4: Information in boldface type is new or modified since the previous edition.

Table 2A. Zone Diameter and MIC Breakpoints for Enterobacterales

Testing Conditions

Medium: Disk diffusion: MHA
Broth dilution: CAMHB; iron-depleted CAMHB for cefiderocol (see Appendix I)[1]
Agar dilution: MHA

Inoculum: Broth culture method or colony suspension, equivalent to a 0.5 McFarland standard; **positive blood culture broth for select antimicrobial agents with disk diffusion (see general comment [5]).**

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®[a] 25922
Pseudomonas aeruginosa ATCC® 27853 (for carbapenems)
***Staphylococcus aureus* ATCC® 25923 (for disk diffusion) or *S. aureus* ATCC® 29213 (for dilution methods) when testing azithromycin against *Salmonella enterica* ser. Typhi *or Shigella* spp.**
Refer to Tables 4A-2 and 5A-2 to select strains for routine QC of ß-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.

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

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,[2] 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 (see the *M02 Disk Diffusion Reading Guide*[3]). 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. enterica* ser. Typhi and *S. enterica* ser. 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 prevention committees, and the antimicrobial stewardship team.

Table 2A. Enterobacterales (Continued)

(4) Intermediate ranges denoted with a ^ for the applicable antimicrobial agents in the drug groups in Tables 2 are based on the known ability of these agents to concentrate in the urine.

(5) Positive blood culture broth can be used as the inoculum for direct disk diffusion testing of select antimicrobial agents (see below) against Enterobacterales as described in Table 3E with a standard incubation of 16 to 18 hours, using current disk diffusion breakpoints in Table 2A. For antimicrobial agents not listed below for Enterobacterales, for other genera, and for shorter direct incubation times, eg, 8 to 10 hours, CLSI has not yet evaluated this direct disk diffusion method.

Antimicrobial Agents
Ampicillin
Aztreonam
Ceftazidime
Ceftriaxone
Tobramycin
Trimethoprim-sulfamethoxazole

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

Table 2A. Enterobacterales (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	**(6)** 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	**(7)** For testing and reporting of *E. coli* urinary tract isolates only.
B-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	**(8)** Breakpoints are based on a dosage regimen of 1.5 g administered every 8 h.
B	Ceftazidime-avibactam	30/20 µg	≥ 21	-	-	≤ 20	≤ 8/4	-	-	≥ 16/4	**(9)** Breakpoints are based on a dosage regimen of 2.5 g every 8 h administered over 2 h. **(10)** Confirmatory MIC testing is indicated for isolates with zones of 20-22 mm to avoid reporting false-susceptible or false-resistant results.
B	Imipenem-relebactam	10/25 µg	≥ 25	-	21-24^	≤ 20	≤ 1/4	-	2/4^	≥ 4/4	**(11) Breakpoints are based on a dosage regimen of 1.25 g administered every 6 h.** **(12) Breakpoints do not apply to the family *Morganellaceae*, which includes but is not limited to the genera *Morganella*, *Proteus*, and *Providencia*.** **(13) Organisms that test susceptible to imipenem are also considered susceptible to imipenem-relebactam. However, organisms that test susceptible to imipenem-relebactam cannot be assumed to be susceptible to imipenem.**

Table 2A. Enterobacterales (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	
β-LACTAM COMBINATION AGENTS (Continued)											
B	Meropenem-vaborbactam	20/10 µg	≥ 18	-	15-17^	≤ 14	≤ 4/8	-	8/8^	≥ 16/8	**(14)** Breakpoints are based on a dosage regimen of 4 g every 8 h administered over 3 h.
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.)											

(15) 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.

(16) 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 prevention purposes. For laboratories that have not implemented the current breakpoints, ESBL testing should be performed as described in Table 3A.

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.

(17) *Enterobacter*, *Klebsiella* (formerly *Enterobacter*) *aerogenes*, *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.

Table 2A. Enterobacterales (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)											
A	Cefazolin	30 µg	≥23	-	20-22	≤19	≤2	-	4	≥8	**(18)** 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 administered every 8 h. See comment (**16**).
U	Cefazolin	30 µg	≥15	-	-	≤14	≤16	-	-	≥32	**(19)** 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 administered every 12 h. See additional information in CEPHEMS (ORAL).
C	Ceftaroline	30 µg	≥23	-	20-22^	≤19	≤0.5	-	1^	≥2	**(20)** Breakpoints are based on a dosage regimen of 600 mg administered every 12 h.
B	Cefepime	30 µg	≥25	19-24	-	≤18	≤2	4-8	-	≥16	**(21)** The breakpoint for susceptible is based on a dosage regimen of 1 g administered 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.

Table 2A. Enterobacterales (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm: S	SDD	I	R	Interpretive Categories and MIC Breakpoints, µg/mL: S	SDD	I	R	Comments
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.) (Continued)											
B B	Cefotaxime or ceftriaxone	30 µg 30 µg	≥ 26 ≥ 23	-	23-25^ 20-22^	≤ 22 ≤ 19	≤ 1 ≤ 1	-	2^ 2^	≥ 4 ≥ 4	**(22)** Breakpoints are based on a dosage regimen of 1 g administered every 24 h for ceftriaxone and 1 g administered every 8 h for cefotaxime. See comment (16).
B	Cefotetan	30 µg	≥ 16	-	13-15^	≤ 12	≤ 16	-	32^	≥ 64	
B	Cefoxitin	30 µg	≥ 18	-	15-17^	≤ 14	≤ 8	-	16^	≥ 32	**(23)** Breakpoints are based on a dosage regimen of at least 8 g per day (eg, 2 g administered every 6 h).
B	Cefuroxime (parenteral)	30 µg	≥ 18	-	15-17^	≤ 14	≤ 8	-	16^	≥ 32	**(24)** Breakpoints are based on a dosage regimen of 1.5 g administered every 8 h. See comment (16).
C	Ceftazidime	30 µg	≥ 21	-	18-20^	≤ 17	≤ 4	-	8^	≥ 16	**(25)** Breakpoints are based on a dosage regimen of 1 g administered every 8 h. See comment (16).
O	Cefamandole	30 µg	≥ 18	-	15-17^	≤ 14	≤ 8	-	16^	≥ 32	See comment (16).
O	Cefmetazole	30 µg	≥ 16	-	13-15^	≤ 12	≤ 16	-	32^	≥ 64	**(26)** Insufficient new data exist to reevaluate breakpoints listed here.
O	Cefonicid	30 µg	≥ 18	-	15-17^	≤ 14	≤ 8	-	16^	≥ 32	See comment (16).
O	Cefoperazone	75 µg	≥ 21	-	16-20	≤ 15	≤ 16	-	32	≥ 64	See comment (16).
O	Ceftizoxime	30 µg	≥ 25	-	22-24^	≤ 21	≤ 1	-	2^	≥ 4	**(27)** Breakpoints are based on a dosage regimen of 1 g administered every 12 h. See comment (16).
O	Moxalactam	30 µg	≥ 23	-	15-22^	≤ 14	≤ 8	-	16-32^	≥ 64	See comment (16).
Inv.	Cefiderocol	30 µg	≥ 16	-	12-15^	≤ 11	≤ 4	-	8^	≥ 16	**(28)** Breakpoints are based on a dosage regimen of 2 g every 8 h administered over 3 h.

Table 2A. Enterobacterales (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)											
B	Cefuroxime	30 µg	≥ 23	-	15-22^	≤ 14	≤ 4		8-16^	≥ 32	See comment (29).
U	Cefazolin (surrogate test for oral cephalosporins and uncomplicated UTIs)	30 µg	≥ 15	-	-	≤ 14	≤ 16	-	-	≥ 32	**(29)** Breakpoints are for cefazolin when used as a surrogate test 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 **tested** 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	**(30)** Do not test *Citrobacter*, *Providencia*, or *Enterobacter* spp. with cefdinir or loracarbef by disk diffusion because false-susceptible results have been reported. See comment (29).
O	Cefaclor	30 µg	≥ 18	-	15-17^	≤ 14	≤ 8	-	16^	≥ 32	See comment (29).
O	Cefdinir	5 µg	≥ 20	-	17-19^	≤ 16	≤ 1	-	2^	≥ 4	See comments (29) and (30).
O	Cefixime	5 µg	≥ 19	-	16-18^	≤ 15	≤ 1	-	2^	≥ 4	**(31)** 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 (29) and (31).
O	Cefprozil	30 µg	≥ 18	-	15-17^	≤ 14	≤ 8	-	16^	≥ 32	**(32)** Do not test *Providencia* spp. with cefprozil by disk diffusion because false-susceptible results have been reported. See comment (29).
Inv.	Cefetamet	10 µg	≥ 18	-	15-17^	≤ 14	≤ 4	-	8^	≥ 16	See comment (31).
Inv.	Ceftibuten	30 µg	≥ 21	-	18-20^	≤ 17	≤ 8	-	16^	≥ 32	**(33)** For testing and reporting of urinary tract isolates only.

Table 2A. Enterobacterales (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	
MONOBACTAMS											
C	Aztreonam	30 µg	≥21	-	18–20^	≤17	≤4	-	8^	≥16	**(34)** Breakpoints are based on a dosage regimen of 1 g administered every 8 h. See comment (16).
CARBAPENEMS											
(35) 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.[4-7] 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 Enterobacterales MIC breakpoints for carbapenems described in M100-S20 (January 2010) should perform the CarbaNP test, mCIM, eCIM, and/or a molecular assay (refer to Tables 3B and 3C for methods) when isolates of Enterobacterales are suspicious for carbapenemase production based on imipenem or meropenem MICs 2–4 µg/mL or ertapenem MIC 2 µg/mL (refer to Tables 3B-1 and 3C-1 for guidance on reporting). After implementing the current breakpoints, these additional tests may not need to be performed other than for epidemiological or infection prevention purposes (ie, it is no longer necessary to edit results for the carbapenems to resistant if a carbapenemase producer is detected). See Appendix H, Table H3 regarding suggestions for reporting when molecular and phenotypic methods are discordant. The following information is provided as background on carbapenemases in Enterobacterales 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	**(36)** Breakpoints are based on a dosage regimen of 500 mg administered every 8 h.
B	Ertapenem	10 µg	≥22	-	19–21^	≤18	≤0.5	-	1^	≥2	**(37)** Breakpoints are based on a dosage regimen of 1 g administered every 24 h.

Table 2A. Enterobacterales (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	
CARBAPENEMS (Continued)											
B	Imipenem	10 µg	≥ 23	-	20-22^	≤ 19	≤ 1	-	2^	≥ 4	**(38)** Breakpoints are based on a dosage regimen of 500 mg administered every 6 h or 1 g every 8 h. **See comment (13).**
B	Meropenem	10 µg	≥ 23	-	20-22^	≤ 19	≤ 1	-	2^	≥ 4	**(39)** Breakpoints are based on a dosage regimen of 1 g administered every 8 h.
LIPOPEPTIDES											
(40) WARNING: Clinical and PK/PD data demonstrate colistin and polymyxin B have limited clinical efficacy, even if an intermediate result is obtained. Alternative agents are strongly preferred. Colistin and polymyxin B should be used in combination with one or more active antimicrobial agents. Consultation with an infectious diseases specialist is recommended. **(41)** Several species are intrinsically resistant to the lipopeptides (colistin and polymyxin B). Refer to Appendix B.											
O	Colistin or polymyxin B		- -	- -	- -	- -	- -	- -	≤ 2 ≤ 2	≥ 4 ≥ 4	**(42)** Colistin (methanesulfonate) should be given with a loading dose and maximum renally adjusted doses (see International Consensus Guidelines[8]). **(43)** Polymyxin B should be given with a loading dose and maximum recommended doses (see International Consensus Guidelines[8]). **(44)** When colistin or polymyxin B is given systemically, neither is likely to be effective for pneumonia. **(45)** For colistin, broth microdilution, CBDE, and CAT MIC methods are acceptable. For polymyxin B, broth microdilution is the only approved method. Disk diffusion and gradient diffusion methods should not be performed (see Table 3D).

Table 2A. Enterobacterales (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											
(46) 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	-	-	-	-	
MACROLIDES											
B	Azithromycin	15 µg	≥13	-	-	≤12	≤16	-	-	≥32	**(47)** *S. enterica* ser. Typhi only: breakpoints are based on MIC distribution data and limited clinical data. **(48) Breakpoints are based on a dosage regimen of 500 mg administered daily.**
			≥16	**-**	**11-15**	**≤10**	**≤8**	**-**	**16**	**≥32**	**(49)** ***Shigella*** **spp. only: azithromycin disk diffusion zones can be hazy and difficult to measure, especially *S. sonnei*. If an isolate has a zone of inhibition that is difficult to measure, an MIC method is recommended. Media source may affect the clarity of the end points for disk diffusion tests.** **See comment (48).**
TETRACYCLINES											
(50) 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	

Table 2A. Enterobacterales (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 Enterobacterales except *Salmonella* spp. (Please refer to Glossary I.)											
B B	Ciprofloxacin Levofloxacin	5 µg 5 µg	≥ 26 ≥ 21	-	22-25^ 17-20^	≤ 21 ≤ 16	≤ 0.25 ≤ 0.5	-	0.5^ 1^	≥ 1 ≥ 2	**(51)** Breakpoints for ciprofloxacin are based on a dosage regimen of 400 mg IV or 500 mg orally administered every 12 h. **(52)** Breakpoints for levofloxacin are based on a dosage regimen of 750 mg administered every 24 h.
O	Cinoxacin	100 µg	≥ 19	-	15-18^	≤ 14	≤ 16	-	32^	≥ 64	See comment **(33)**.
O	Enoxacin	10 µg	≥ 18	-	15-17^	≤ 14	≤ 2	-	4^	≥ 8	See comment **(33)**.
O	Gatifloxacin	5 µg	≥ 18	-	15-17^	≤ 14	≤ 2	-	4^	≥ 8	
O	Gemifloxacin	5 µg	≥ 20	-	16-19	≤ 15	≤ 0.25	-	0.5	≥ 1	**(53)** 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 **(33)**.
O	Norfloxacin	10 µg	≥ 17	-	13-16	≤ 12	≤ 4	-	8	≥ 16	See comment **(33)**.
O	Ofloxacin	5 µg	≥ 16	-	13-15^	≤ 12	≤ 2	-	4^	≥ 8	
Inv.	Fleroxacin	5 µg	≥ 19	-	16-18^	≤ 15	≤ 2	-	4^	≥ 8	
QUINOLONES AND FLUOROQUINOLONES for *Salmonella* spp. (Please refer to Glossary I.)											
(54) For testing and reporting of *Salmonella* spp. (including *S. enterica* ser. Typhi and *S. enterica* ser. Paratyphi A-C). Routine susceptibility testing is not indicated for nontyphoidal *Salmonella* spp. isolated from intestinal sources. **(55)** 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. **(56)** No single test detects resistance resulting from all possible fluoroquinolone resistance mechanisms that have been identified in *Salmonella* spp.										

Table 2A. Enterobacterales (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.) (Continued)											
B	Ciprofloxacin	5 µg	≥ 31	-	21-30^	≤ 20	≤ 0.06	-	0.12-0.5 ^	≥ 1	**(57)** 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.
B	Levofloxacin	-	-	-	-	-	≤ 0.12	-	0.25-1^	≥ 2	
O	Ofloxacin	-	-	-	-	-	≤ 0.12	-	0.25-1^	≥ 2	
Inv.	Pefloxacin (surrogate test for ciprofloxacin)	5 µg	≥ 24	-	-	≤ 23	-	-	-	-	**(58)** 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 (56).
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	**(59)** 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	**(60)** Not routinely reported on isolates from the urinary tract.
FOSFOMYCINS											
U	Fosfomycin	200 µg	≥ 16	-	13-15	≤ 12	≤ 64	-	128	≥ 256	**(61)** Disk diffusion and MIC breakpoints apply only to *E. coli* urinary tract isolates and should not be extrapolated to other species of Enterobacterales. **(62)** The 200-µg fosfomycin disk contains 50 µg of glucose-6-phosphate. **(63)** 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.

Table 2A. Enterobacterales (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	
NITROFURANS											
U	Nitrofurantoin	300 µg	≥17	-	15-16	≤14	≤32	-	64	≥128	

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; CAT, colistin agar test; CBDE, colistin broth disk elution; eCIM, EDTA-modified carbapenem inactivation method; ESBL, extended-spectrum β-lactamase; I, intermediate; IV, intravenous; 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.
Symbol: ^, designation for agents that have the potential to concentrate in the urine.

Footnote

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

References for Table 2A

1 Hackel MA, Tsuji M, Yamano Y, Echols R, Karlowsky JA, Sahm DF. Reproducibility of broth microdilution MICs for the novel siderophore cephalosporin, cefiderocol, determined using iron-depleted cation-adjusted Mueller-Hinton broth. *Diagn Microbiol Infect Dis*. 2019;94(4):321-325.

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

3 CLSI. *M02 Disk Diffusion Reading Guide*. 1st ed. CLSI quick guide M02QG. Clinical and Laboratory Standards Institute; 2018.

4 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.

5 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.

6 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.

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

8 Tsuji BT, Pogue JM, Zavascki AP, et al. International consensus guidelines for the optimal use of the polymyxins: endorsed by the American College of Clinical Pharmacy (ACCP), European Society of Clinical Microbiology and Infectious Diseases (ESCMID), Infectious Diseases Society of America (IDSA), International Society for Anti-Infective Pharmacology (ISAP), Society of Critical Care Medicine (SCCM), and Society of Infectious Diseases Pharmacists (SIDP). *Pharmacotherapy*. 2019;39(1):10-39.

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

Testing Conditions

Medium: Disk diffusion: MHA
Broth dilution: CAMHB; iron-depleted CAMHB for cefiderocol (see Appendix I)[1]
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®[a] 27853

Refer to Tables 4A-2 and 5A-2 to select strains for routine QC of ß-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.

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,[2] 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 (see the *M02 Disk Diffusion Reading Guide*[3]). 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 prevention committees, and the antimicrobial stewardship team.

(5) Intermediate ranges denoted with a ^ for the applicable antimicrobial agents in the drug groups in Tables 2 are based on the known ability of these agents to concentrate in the urine.

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

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	**(6)** Breakpoints for piperacillin (alone or with tazobactam) are based on a piperacillin dosage regimen of at least 3 g administered 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	**(7)** Breakpoints for piperacillin (alone or with tazobactam) are based on a piperacillin dosage regimen of at least 3 g administered every 6 h.
B	Ceftazidime-avibactam	30/20 µg	≥21	-	≤20	≤8/4	-	≥16/4	**(8)** Breakpoints are based on a dosage regimen of 2.5 g administered every 8 h over 2 h.
B	Ceftolozane-tazobactam	30/10 µg	≥21	17-20^	≤16	≤4/4	8/4^	≥16/4	**(9)** Breakpoints are based on a dosage regimen of 1.5 g administered every 8 h.
B	**Imipenem-relebactam**	**10/25 µg**	**≥23**	**20-22^**	**≤19**	**≤2/4**	**4/4^**	**≥8/4**	**(10) Breakpoints are based on a dosage regimen of 1.25 g administered every 6 h.** **(11) Organisms that test susceptible to imipenem are also considered susceptible to imipenem-relebactam. However, organisms that test susceptible to imipenem-relebactam cannot be assumed to be susceptible to imipenem.**
O	Ticarcillin-clavulanate	75/10 µg	≥24	16-23^	≤15	≤16/2	32/2-64/2^	≥128/2	**(12)** Breakpoints for ticarcillin (alone or with clavulanate) are based on a ticarcillin dosage regimen of at least 3 g administered 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	**(13)** Breakpoints are based on a dosage regimen of 1 g administered every 6 h or 2 g administered every 8 h.
B	Cefepime	30 µg	≥18	15-17^	≤14	≤8	16^	≥32	**(14)** Breakpoints are based on a dosage regimen of 1 g administered every 8 h or 2 g administered every 12 h.
Inv.	Cefiderocol	30 µg	≥18	13-17^	≤12	≤4	8^	≥16	**(15)** Breakpoints are based on a dosage regimen of 2 g every 8 h administered over 3 h.
MONOBACTAMS									
B	Aztreonam	30 µg	≥22	16-21^	≤15	≤8	16^	≥32	**(16)** Breakpoints are based on a dosage regimen of 1 g administered every 6 h or 2 g administered every 8 h.

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	
CARBAPENEMS									
B	Doripenem	10 µg	≥19	16-18^	≤15	≤2	4^	≥8	**(17)** Breakpoints for doripenem are based on a dosage regimen of 500 mg administered every 8 h.
	Imipenem	10 µg	≥19	16-18^	≤15	≤2	4^	≥8	**(18)** Breakpoints for imipenem are based on a dosage regimen of 1 g administered every 8 h or 500 mg administered every 6 h. **See comment (11).**
	Meropenem	10 µg	≥19	16-18^	≤15	≤2	4^	≥8	**(19)** Breakpoints for meropenem are based on a dosage regimen of 1 g administered every 8 h.
LIPOPEPTIDES									
(20) WARNING: Clinical and PK/PD data demonstrate colistin and polymyxin B have limited clinical efficacy, even if an intermediate result is obtained. Alternative agents are strongly preferred. Colistin and polymyxin B should be used in combination with one or more active antimicrobial agents. Consultation with an infectious diseases specialist is recommended.									
O	Colistin or polymyxin B	- -	- -	- -	- -	- -	≤2 ≤2	≥4 ≥4	**(21)** Colistin (methanesulfonate) should be given with a loading dose and maximum renally adjusted doses (see International Consensus Guidelines[4]). **(22)** Polymyxin B should be given with a loading dose and maximum recommended doses (see International Consensus Guidelines[4]). **(23)** When colistin or polymyxin B is given systemically, neither is likely to be effective for pneumonia. **(24)** For colistin, broth microdilution, CBDE, and CAT MIC methods are acceptable. For polymyxin B, broth microdilution is the only approved method. Disk diffusion and gradient diffusion methods should not be performed (see Table 3D).

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	
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	≥ 25	19-24^	≤ 18	≤ 0.5	1^	≥ 2	**(25)** Breakpoints are based on a dosage regimen of 400 mg IV administered every 8 h.
B	Levofloxacin	5 µg	≥ 22	15-21^	≤ 14	≤ 1	2^	≥ 4	**(26)** Breakpoints are based on a dosage regimen of 750 mg administered every 24 h.
O	Lomefloxacin	10 µg	≥ 22	19-21^	≤ 18	≤ 2	4^	≥ 8	**(27)** For testing and reporting of urinary tract isolates only.
O	Norfloxacin	10 µg	≥ 17	13-16	≤ 12	≤ 4	8	≥ 16	See comment (27).
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; CAT, colistin agar test; CBDE, colistin broth disk elution; I, intermediate; IV, intravenous; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; PK/PD, pharmacokinetic/pharmacodynamic; QC, quality control; R, resistant; S, susceptible.

Symbol: ^, designation for agents that have the potential to concentrate in the urine.

<u>Footnote</u>

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

References for Table 2B-1

[1] Hackel MA, Tsuji M, Yamano Y, Echols R, Karlowsky JA, Sahm DF. Reproducibility of broth microdilution MICs for the novel siderophore cephalosporin, cefiderocol, determined using iron-depleted cation-adjusted Mueller-Hinton broth. *Diagn Microbiol Infect Dis*. 2019;94(4):321-325.

[2] CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.

[3] CLSI. *M02 Disk Diffusion Reading Guide*. 1st ed. CLSI quick guide M02QG. Clinical and Laboratory Standards Institute; 2018.

[4] Tsuji BT, Pogue JM, Zavascki AP, et al. International consensus guidelines for the optimal use of the polymyxins: endorsed by the American College of Clinical Pharmacy (ACCP), European Society of Clinical Microbiology and Infectious Diseases (ESCMID), Infectious Diseases Society of America (IDSA), International Society for Anti-Infective *Pharmacology* (ISAP), Society of Critical Care Medicine (SCCM), and Society of Infectious Diseases Pharmacists (SIDP). *Pharmacotherapy*. 2019;39(1):10-39.

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

Testing Conditions

Medium: Disk diffusion: MHA
Broth dilution: CAMHB; iron-depleted CAMHB for cefiderocol (see Appendix I)[1]
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®[a] 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 ß-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.

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,[2] 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 (see the *M02 Disk Diffusion Reading Guide*[3]). 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.

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	
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	
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	
Inv.	Cefiderocol	30 µg	≥15	11-14	≤10	≤4	8	≥16	**(2)** Breakpoints are based on a dosage regimen of 2 g every 8 h administered over 3 h.
CARBAPENEMS									
A	Doripenem	10 µg	≥18	15-17	≤14	≤2	4	≥8	**(3)** Breakpoints for doripenem are based on a dosage regimen of 500 mg administered every 8 h.
A	Imipenem	10 µg	≥22	19-21	≤18	≤2	4	≥8	**(4)** Breakpoints for imipenem are based on a dosage regimen of 500 mg administered every 6 h.
A	Meropenem	10 µg	≥18	15-17	≤14	≤2	4	≥8	**(5)** Breakpoints for meropenem are based on a dosage regimen of 1 g administered every 8 h or 500 mg administered every 6 h.

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	
LIPOPEPTIDES									
(6) WARNING: Clinical and PK/PD data demonstrate colistin and polymyxin B have limited clinical efficacy, even if an intermediate result is obtained. Alternative agents are strongly preferred. Colistin and polymyxin B should be used in combination with one or more active antimicrobial agents. Consultation with an infectious diseases specialist is recommended.									
O	Colistin or polymyxin B	- -	- -	- -	- -	- -	≤2 ≤2	≥4 ≥4	**(7)** Colistin (methanesulfonate) should be given with a loading dose and maximum renally adjusted doses (see International Consensus Guidelines[4]). **(8)** Polymyxin B should be given with a loading dose and maximum recommended doses (see International Consensus Guidelines[4]). **(9)** When colistin or polymyxin B is given systemically, the drug is unlikely to be effective for pneumonia. **(10)** The only approved MIC method is broth microdilution. CBDE, CAT, disk diffusion, and gradient diffusion should not be performed. **(11)** Applies to *A. baumannii* complex only.
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									
(12) 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	

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; CAT, colistin agar test; CBDE, colistin broth elution test; I, intermediate; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; PK/PD, pharmacokinetic/pharmacodynamic; QC, quality control; R, resistant; S, susceptible.

Footnote

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

References for Table 2B-2

1 Hackel MA, Tsuji M, Yamano Y, Echols R, Karlowsky JA, Sahm DF. Reproducibility of broth microdilution MICs for the novel siderophore cephalosporin, cefiderocol, determined using iron-depleted cation-adjusted Mueller-Hinton broth. *Diagn Microbiol Infect Dis*. 2019;94(4):321-325.

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

3 CLSI. *M02 Disk Diffusion Reading Guide*. 1st ed. CLSI quick guide M02QG. Clinical and Laboratory Standards Institute; 2018.

4 Tsuji BT, Pogue JM, Zavascki AP, et al. International consensus guidelines for the optimal use of the polymyxins: endorsed by the American College of Clinical Pharmacy (ACCP), European Society of Clinical Microbiology and Infectious Diseases (ESCMID), Infectious Diseases Society of America (IDSA), International Society for Anti-Infective Pharmacology (ISAP), Society of Critical Care Medicine (SCCM), and Society of Infectious Diseases Pharmacists (SIDP). *Pharmacotherapy*. 2019;39(1):10-39.

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®[a] 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.

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 (see the *M02 Disk Diffusion Reading Guide*[2]). 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.

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.

<u>**Footnote**</u>

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

References for Table 2B-3

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

[2] CLSI. *M02 Disk Diffusion Reading Guide*. 1st ed. CLSI quick guide M02QG. Clinical and Laboratory Standards Institute; 2018.

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

Testing Conditions

Medium: Disk diffusion: MHA
Broth dilution: CAMHB; iron-depleted CAMHB for cefiderocol (see Appendix I)[1]
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®[a] 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.

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,[2] 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 (see the *M02 Disk Diffusion Reading Guide*[3]). 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.

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	
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	
Inv.	Cefiderocol	30 µg	≥17	13-16	≤12	≤ 4	8	≥ 16	**(2)** Breakpoints are based on a dosage regimen of 2 g every 8 h administered over 3 h.
TETRACYCLINES									
A	Minocycline	30 µg	≥19	15-18	≤14	≤4	8	≥16	
FLUOROQUINOLONES									
A	Levofloxacin	5 µg	≥17	14-16	≤13	≤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	**(3)** 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.

Footnote

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

References for Table 2B-4

[1] Hackel MA, Tsuji M, Yamano Y, Echols R, Karlowsky JA, Sahm DF. Reproducibility of broth microdilution MICs for the novel siderophore cephalosporin, cefiderocol, determined using iron-depleted cation-adjusted Mueller-Hinton broth. *Diagn Microbiol Infect Dis*. 2019;94(4):321-325.

[2] CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.

[3] CLSI. *M02 Disk Diffusion Reading Guide*. 1st ed. CLSI quick guide M02QG. Clinical and Laboratory Standards Institute; 2018.

Table 2B-5. MIC Breakpoints for Other Non-Enterobacterales (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 Table 5A-1 for acceptable QC ranges)

Escherichia coli ATCC®[a] 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 ß-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.

General Comments

(1) Other non-Enterobacterales include *Pseudomonas* spp. and other nonfastidious, glucose-nonfermenting, gram-negative bacilli but exclude *P. aeruginosa*, *Acinetobacter* spp., *B. cepacia* complex, and *S. maltophilia* (refer to Tables 2B-2, 2B-3, and 2B-4, respectively). Recommendations for testing and reporting *Aeromonas hydrophila* complex, *Burkholderia mallei*, *Burkholderia pseudomallei*, and *Vibrio* spp. (including *V. cholerae)* are found in CLSI document M45.[1]

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

Table 2B-5. Other Non-Enterobacterales (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	

Table 2B-5. Other Non-Enterobacterales (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.

Footnote

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

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. 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. **NOTE:** 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 (for cefoxitin when testing *Staphylococcus* spp., **except** *S. aureus, S. lugdunensis*, *S. pseudintermedius*, and *S. schleiferi*) Dilution methods: 16–20 hours; 24 hours for oxacillin and vancomycin Testing at temperatures above 35°C may not detect methicillin (oxacillin)-resistant staphylococci (MRS).

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

Disk diffusion:
S. aureus ATCC®[a] 25923

Dilution methods:
S. aureus ATCC® 29213

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.

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 (see the *M02 Disk Diffusion Reading Guide*[2]). 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) ***S. aureus*** **complex consists of the coagulase-positive species** ***S. aureus*****,** ***Staphylococcus argenteus*****, and** ***Staphylococcus schweitzeri*****. If** ***S. argenteus*** **is identified by MALDI-TOF MS or sequencing, it is recommended that it be reported as "*S. aureus* complex (*S. argenteus*)," and** ***S. aureus*** **phenotypic testing method recommendations, breakpoints, and interpretive categories should be used. Human infections with** ***S. schweitzeri*** **have yet to be reported.**[3]

Table 2C. Staphylococcus spp. (Continued)

(3) 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,[4] 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 compared with the control (see M07,[4] Figure 5).

(4) 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).

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

(6) Most methicillin (oxacillin) resistance is mediated by *mecA*, encoding PBP2a (also called PBP2′). **Testing for *mecA* and PBP2a are the most definitive tests for detection of methicillin (oxacillin) resistance for *Staphylococcus* spp.** Isolates that test positive for *mecA* or PBP2a **or resistant by any of the recommended phenotypic methods** should be reported as methicillin (oxacillin) resistant (see Appendix H **and table below**).

Detection of methicillin (oxacillin) resistance in staphylococci is achieved by using specific methods as listed in Table 2C and further described in Tables **3G-1 and 3G-2.**

Organism	Phenotypic Methods for Detection of Methicillin (Oxacillin)-Resistant *Staphylococcus* spp.				
	Cefoxitin MIC	Cefoxitin disk diffusion	Oxacillin MIC	Oxacillin disk diffusion	Oxacillin salt agar
S. aureus	Yes (16-20 h)	Yes (16-18 h)	Yes (24 h)	No	Yes (24 h)
S. lugdunensis	Yes (16-20 h)	Yes (16-18 h)	Yes (24 h)	No	No
S. epidermidis	No	Yes (24 h)	Yes (24 h)	Yes (16-18 h)	No
S. pseudintermedius	No	No	Yes (24 h)	Yes (16-18 h)	No
S. schleiferi	No	No	Yes (24 h)	Yes (16-18 h)	No
Staphylococcus spp. (not listed above **or not identified to the species level)**	No	Yes[a] (24 h)	Yes[a] (24 h)	No	No

Abbreviations: h, hour(s); MIC, minimal inhibitory concentration; MRS, methicillin (oxacillin)-resistant staphylococci; PBP2a, penicillin-binding protein 2a.
[a] For isolates **that fall into the category** of *Staphylococcus* spp **(not listed above or not identified to the species level)** from serious infections for which the oxacillin MICs are **1**–2 µg/mL, testing for *mecA* or PBP2a should be considered, **because these are the most definitive tests for detection of methicillin (oxacillin) resistance** (see comment **[18]**). **Recent data suggest that the cefoxitin disk diffusion test may not perform reliably for all species (eg, *S. haemolyticus*) that fall into the category of "*Staphylococcus* spp. (not listed above or not identified to the species level)."**[5]

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

Mechanisms of methicillin (oxacillin) resistance other than *mecA* are rare and include a novel *mecA* homologue, *mecC*.[6] MICs for strains with *mecC* are typically cefoxitin resistant and oxacillin susceptible; *mecC* resistance cannot be detected by tests directed at *mecA* or PBP2a.

(7) MRS, as defined by cefoxitin or oxacillin testing, as appropriate to the species, are considered resistant to other β-lactam agents, ie, penicillins, β-lactam combination agents, cephems (with the exception of ceftaroline), 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.

(8) For tests for β-lactamase production, methicillin (oxacillin) resistance and *mecA*-mediated methicillin (oxacillin) resistance using cefoxitin, reduced susceptibility to vancomycin, ICR, and high-level mupirocin resistance (*S. aureus* only), refer to Tables **3F, 3G-1, 3G-2, 3H,** and **3J,** respectively.

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

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

Test/Report Group	Antimicrobial Agent	*Staphylococcus* spp. Indications	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	
PENICILLINASE-LABILE PENICILLINS												
(9) 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. **(10)** Penicillin should be used to test the susceptibility of all staphylococci to 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 *blaZ* β-lactamase gene may be considered. See Table **3F.**												
A	Penicillin	All staphylococci	10 units	≥29	-	-	≤28	≤0.12		-	≥0.25	**(11)** For methicillin (oxacillin)-resistant staphylococci, report penicillin as resistant or do not report.
PENICILLINASE-STABLE PENICILLINS												

(12) Cefoxitin is tested as a surrogate for oxacillin for some species of *Staphylococcus*. Isolates that test resistant by cefoxitin or oxacillin, when using the appropriate test method for the species, should be reported as methicillin (oxacillin) resistant. If testing only cefoxitin, report as methicillin (oxacillin) susceptible or resistant based on the cefoxitin result.

(13) 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, methicillin (oxacillin)-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)

Methicillin (oxacillin)-resistant staphylococci are resistant to all currently available β-lactam antimicrobial agents, with the exception of ceftaroline. 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 ceftaroline, is not advised. See general comments (6) and (7).

Additional explanation on the use of cefoxitin for prediction of *mecA*-mediated methicillin (oxacillin) resistance can be found in Subchapter 3.12 of M07[4] and Subchapter 3.9 of M02.[1]

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

Test/ Report Group	Antimicrobial Agent	*Staphylococcus* spp. Indications	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm: S	SDD	I	R	Interpretive Categories and MIC Breakpoints, µg/mL: S	SDD	I	R	Comments
PENICILLINASE-STABLE PENICILLINS (Continued)												
A	Oxacillin	*S. aureus* and *S. lugdunensis*	-	-	-	-	-	≤ 2 (oxacillin)	-	-	≥ 4 (oxacillin)	**(14)** Oxacillin disk testing is not reliable for *S. aureus* and *S. lugdunensis*. **(15)** For isolates of S. *aureus* that do not grow well on CAMHB or unsupplemented MHA (eg, small-colony variants), testing on other media (eg, BMHA) does not reliably detect *mecA*-mediated resistance. 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. See general comments (6) and (7) and comments (9), (12), and (13).
			30 µg cefoxitin (surrogate test for oxacillin)	≥ 22	-	-	≤ 21	≤ 4 (cefoxitin)	-	-	≥ 8 (cefoxitin)	
A	Oxacillin	*S. epidermidis*	1 µg oxacillin	≥ 18 (oxacillin)	–	–	≤ 17 (oxacillin)	**≤ 0.5** (oxacillin)	–	–	≥ 1 (oxacillin)	See general comments (6) and (7) and comments (9), (12), and (13).
			30 µg cefoxitin (surrogate test for oxacillin)	≥ 25 (cefoxitin)	–	–	≤ 24 (cefoxitin)	–	–	–	–	**(16)** Cefoxitin MIC testing is not reliable for detecting *mecA*-mediated resistance in *S. epidermidis*.
		S. pseudintermedius and *S. schleiferi*	1 µg oxacillin	≥ 18	–	-	≤ 17	**≤ 0.5**	–	-	**≥ 1**	**(17)** Neither cefoxitin MIC nor cefoxitin disk tests are reliable for detecting *mecA*-mediated resistance in *S. pseudintermedius* and *S. schleiferi*. See general comments (6) and (7) and comments (9), (12), and (13).

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

Test/ Report Group	Antimicrobial Agent	*Staphylococcus* spp. Indications	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	
PENICILLINASE-STABLE PENICILLINS (Continued)												
A	Oxacillin	*Staphylococcus* spp., except: *S. aureus* *S. lugdunensis* *S. epidermidis* *S. pseudintermedius* *S. schleiferi*	30 µg cefoxitin (surrogate test for oxacillin)	≥ 25 (cefoxitin)	–	-	≤ 24 (cefoxitin)	**≤ 0.5** (oxacillin)	–	-	≥ **1** (oxacillin)	**(18)** Oxacillin MIC breakpoints may overcall resistance, and some isolates for which the oxacillin MICs are **1**-2 µg/mL may be *mecA* negative. Isolates from serious infections for which oxacillin MICs are **1**-2 µg/mL may be tested for *mecA* or for PBP2a. Isolates that test *mecA* or PBP2a negative should be reported as methicillin (oxacillin) susceptible. See general comments (6) and (7) and comments (9), (12), and (13).
CEPHEMS (PARENTERAL)												
B	Ceftaroline	*S. aureus*, including MRSA	30 µg	≥ 25	20-24		≤ 19	≤ 1	2-4	–	≥ 8	**(19)** The breakpoint for susceptible is based on a dosage regimen of 600 mg administered every 12 h. **(20)** The breakpoint for SDD is based on a dosage of 600 mg every 8 h administered over 2 h.

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

Test/Report Group	Antimicrobial Agent	*Staphylococcus* spp. Indications	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	
GLYCOPEPTIDES												
(21) MIC tests should be performed to determine the susceptibility of all isolates of stphylococci 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 *Staphylococcus* spp. other than *S. aureus*, all of which give similar size zones of inhibition.												
B	Vancomycin	*S. aureus*	-	-	-	-	-	≤2	-	4-8	≥16	**(22)** For *S. aureus*, vancomycin-susceptible isolates may become vancomycin intermediate during the course of prolonged therapy. **(23)** Send any *S. aureus* for which the vancomycin is ≥8 µg/mL to a referral laboratory. See Appendix A. Also refer to Table **3G-1** for *S. aureus*, Subchapter 3.12 in M07,[4] and Subchapter 3.9 in M02.[1]
		Staphylococcus spp. other than *S. aureus*	-	-	-	-	-	≤4	-	8-16	≥32	See comment (20). **(24)** Send any *Staphylococcus* spp. other than *S. aureus* for which the vancomycin MIC is ≥32 µg/mL to a referral laboratory. See Appendix A. See also Subchapter 3.12 in M07[4] and Subchapter 3.9 in M02.[1]
LIPOGLYCOPEPTIDES												
C	Dalbavancin	*S. aureus*, including MRSA	-	-	-	-	-	≤0.25	-	-	-	
C	Oritavancin		-	-	-	-	-	≤0.12	-	-	-	
C	Telavancin		-	-	-	-	-	≤0.12	-	-	-	
Inv.	Teicoplanin	All staphylococci	-	-	-	-	-	≤8	-	16	≥32	
LIPOPEPTIDES												
B	Daptomycin	All staphylococci	-	-	-	-	-	≤1	-	-	-	**(25)** Daptomycin should not be reported for isolates from the respiratory tract.
AMINOGLYCOSIDES												
(26) For staphylococci that test susceptible, gentamicin is used only in combination with other active agents that test susceptible.												
C	Gentamicin	All staphylococci	10 µg	≥15	-	13-14	≤12	≤4	-	8	≥16	

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

Test/ Report Group	Antimicrobial Agent	*Staphylococcus* spp. Indications	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	
MACROLIDES												
(27) Not routinely reported on organisms isolated from the urinary tract.												
A	Azithromycin	All staphylococci	15 µg	≥18	-	14-17	≤13	≤2	-	4	≥8	
A	or clarithromycin		15 µg	≥18		14-17	≤13	≤2		4	≥8	
A	or erythromycin		15 µg	≥23		14-22	≤13	≤0.5		1-4	≥8	
O	Dirithromycin		15 µg	≥19	-	16-18	≤15	≤2	-	4	≥8	
TETRACYCLINES												
(28) 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	All staphylococci	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 (27).
FLUOROQUINOLONES												
(29) *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	All staphylococci	5 µg	≥21	-	16-20	≤15	≤1	-	2	≥4	
C	or 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	**(30)** 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 (30).
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	
NITROFURANS												
U	Nitrofurantoin	All staphylococci	300 µg	≥17	-	15-16	≤14	≤32	-	64	≥128	

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

Test/ Report Group	Antimicrobial Agent	*Staphylococcus* spp. Indications	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	
LINCOSAMIDES												
A	Clindamycin	All staphylococci	2 µg	≥21	-	15-20	≤14	≤0.5	-	1-2	≥4	**(31)** For isolates that test erythromycin resistant and clindamycin susceptible or intermediate, testing for ICR by disk diffusion using the D-zone test or by broth microdilution is required before reporting clindamycin (see Table **3I**, Subchapter 3.9 in M02,[1] and Subchapter 3.12 in M07[4]). See comment (27).
FOLATE PATHWAY ANTAGONISTS												
A	Trimethoprim-sulfamethoxazole	All staphylococci	1.25/23.75 µg	≥16	-	11-15	≤10	≤2/38	-	-	≥4/76	
U	Sulfonamides	All staphylococci	250 or 300 µg	≥17	-	13-16	≤12	≤256	-	-	≥512	**(32)** Sulfisoxazole can be used to represent any of the currently available sulfonamide preparations.
U	Trimethoprim	All staphylococci	5 µg	≥16	-	11-15	≤10	≤8	-	-	≥16	
PHENICOLS												
C	Chloramphenicol	All staphylococci	30 µg	≥18	-	13-17	≤12	≤8	-	16	≥32	See comment (27).
ANSAMYCINS												
B	Rifampin	All staphylococci	5 µg	≥20	-	17-19	≤16	≤1	-	2	≥4	**(33)** ***Rx:*** Rifampin should not be used alone for antimicrobial therapy.
STREPTOGRAMINS												
O	Quinupristin-dalfopristin	*S. aureus*	15 µg	≥19	-	16-18	≤15	≤1	-	2	≥4	**(34)** For reporting against methicillin (oxacillin)-susceptible *S. aureus*.

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

Test/ Report Group	Antimicrobial Agent	*Staphylococcus* spp. Indications	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	
OXAZOLIDINONES												
(35) *S. aureus* that test susceptible to linezolid by MIC are also considered susceptible to tedizolid. However, some organisms that test resistant to linezolid may be susceptible to tedizolid.												
B	Linezolid	All staphylococci	30 µg	≥21	-	-	≤20	≤ 4	-	-	≥8	**(36)** 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	*S. aureus*, including MRSA	-	-	-	-	-	≤0.5	-	1	≥2	
PLEUROMUTILINS												
B	Lefamulin	*S. aureus*	20 µg	≥23	-	-	-	≤0.25	-	-	-	**(37) The breakpoints for susceptible are based on a dosage regimen of 150 mg IV or 600 mg orally administered every 12 h.** **(38) Not routinely reported on organisms isolated from the urinary tract.**

Abbreviations: ATCC®, American Type Culture Collection; BMHA, blood Mueller-Hinton agar; CAMHB, cation-adjusted Mueller-Hinton broth; I, intermediate; ICR, inducible clindamycin resistance; IV, intravenous; **MALDI-TOF MS; matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry**; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; MRS, methicillin (oxacillin)-resistant staphylococci; MRSA, methicillin (oxacillin)-resistant *S. aureus;* PBP2a, penicillin-binding protein 2a; PCR, polymerase chain reaction; QC, quality control; R, resistant; S, susceptible; SDD, susceptible-dose dependent; UTI, urinary tract infection.

Footnote

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

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

References for Table 2C

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

2 CLSI. *M02 Disk Diffusion Reading Guide*. 1st ed. CLSI quick guide M02QG. Clinical and Laboratory Standards Institute; 2018.

3 **Becker K, Schaumburg F, Kearns A, et al. Implications of Identifying the recently defined members of the *Staphylococcus aureus* complex S. *argenteus* and *S. schweitzeri*: a position paper of members of the ESCMID Study Group for Staphylococci and Staphylococcal Diseases (ESGS). *Clin Microbiol Infect*. 2019;25(9):1064-1070.**

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

5 **Humphries RM, Magnano P, Burnham CA, et al. Evaluation of surrogate tests for the presence of *mecA*-mediated methicillin resistance in *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus capitis* and *Staphylococcus warneri*. *J. Clin Microbiol*. 2020;59(1):e02290-20.**

6 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®[a] 25923

Dilution methods:
E. faecalis ATCC® 29212

Refer to Tables 4A-2 and 5A-2 to select strains for routine QC of ß-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.

Refer to Tables **3H** and **3K** 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 (see the *M02 Disk Diffusion Reading Guide*[2]). 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,[3] 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 **3K**).

(5) Intermediate ranges denoted with a ^ for the applicable antimicrobial agents in the drug groups in Tables 2 are based on the known ability of these agents to concentrate in the urine.

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

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	SDD	I	R	
PENICILLINS										
A	Penicillin	10 units	≥15	-	≤14	≤8	-	-	≥16	**(6)** 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*. **(7)** 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. **(8)** ***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 enterococci. **(9)** 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).
A	Ampicillin	10 µg	≥17	-	≤16	≤8	-	-	≥16	

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	SDD	I	R	
GLYCOPEPTIDES										
B	Vancomycin	30 µg	≥17	15-16	≤14	≤4	-	8-16	≥32	**(10)** 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.[3] 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 **3H**. See general comment (4) and comment (8).
LIPOGLYCOPEPTIDES										
C	Dalbavancin	-	-	-	-	≤0.25	-	-	-	**(11)** For reporting against vancomycin-susceptible *E. faecalis*.
C	Oritavancin	-	-	-	-	≤0.12	-	-	-	See comment (11).
C	Telavancin	-	-	-	-	≤0.25	-	-	-	See comment (11).
Inv.	Teicoplanin	30 µg	≥14	11-13	≤10	≤8	-	16	≥32	
LIPOPEPTIDES										
B	Daptomycin *E. faecium* only	-	-	-	-	-	≤ 4	-	≥ 8	**(12)** Daptomycin should not be reported for isolates from the respiratory tract. **(13)** The breakpoint for SDD is based on a dosage regimen of 8-12 mg/kg administered every 24 h and is intended for serious infections due to *E. faecium*. Consultation with an infectious diseases specialist is recommended.
B	Daptomycin *Enterococcus* spp. other than *E. faecium*	-	-	-	-	≤2	-	4	≥ 8	**(14)** The breakpoint for susceptible is based on a dosage regimen of 6 mg/kg administered every 24 h. See comment (12).
MACROLIDES										
O	Erythromycin	15 µg	≥23	14-22	≤13	≤0.5	-	1-4	≥8	**(15)** Not routinely reported on isolates from the urinary tract.

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	SDD	I	R	
TETRACYCLINES										
(16) 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 U	Ciprofloxacin Levofloxacin	5 µg 5 µg	≥21 ≥17	16-20^ 14-16^	≤15 ≤13	≤1 ≤2	-	2^ 4^	≥4 ≥8	
O	Gatifloxacin	5 µg	≥18	15-17^	≤14	≤2	-	4^	≥8	
O	Norfloxacin	10 µg	≥17	13-16	≤12	≤4	-	8	≥16	**(17)** For testing and reporting of urinary tract isolates only.
NITROFURANS										
U	Nitrofurantoin	300 µg	≥17	15-16	≤14	≤32	-	64	≥128	
ANSAMYCINS										
O	Rifampin	5 µg	≥20	17-19	≤16	≤1	-	2	≥4	**(18)** ***Rx:*** Rifampin should not be used alone for antimicrobial therapy.
FOSFOMYCINS										
U	Fosfomycin	200 µg	≥16	13-15	≤12	≤64	-	128	≥256	**(19)** For testing and reporting of *E. faecalis* urinary tract isolates only. **(20)** 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. **(21)** The 200-µg fosfomycin disk contains 50 µg glucose-6-phosphate.
PHENICOLS										
O	Chloramphenicol	30 µg	≥18	13-17	≤12	≤8	-	16	≥32	See comment (15).
STREPTOGRAMINS										
O	Quinupristin-dalfopristin	15 µg	≥19	16-18	≤15	≤1	-	2	≥4	**(22)** For reporting against vancomycin-resistant *Enterococcus faecium*.
OXAZOLIDINONES										
(23) *E. faecalis* that test susceptible to linezolid by MIC are also considered susceptible to tedizolid. However, some organisms that are intermediate or resistant to linezolid may be susceptible to tedizolid.										
B	Linezolid	30 µg	≥23	21-22	≤20	≤2	-	4	≥8	
B	Tedizolid	-	-	-	-	≤0.5	-	-	-	**(24)** 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; SDD, susceptible-dose dependent.

Symbol: ^, designation for agents that have the potential to concentrate in the urine.

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

Footnote

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

References for Table 2D

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

2 CLSI. *M02 Disk Diffusion Reading Guide*. 1st ed. CLSI quick guide M02QG. Clinical and Laboratory Standards Institute; 2018.

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

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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 general 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®[a] 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.

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.

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 NAD dissolved in 10 mL distilled water, filter sterilized) aseptically.

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

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	
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).
C	**Ceftolozane-tazobactam**	**-**	**-**	**-**	**-**	**≤ 0.5/4**	**-**	**-**	**(9) The susceptible breakpoint is based on a dosage regimen of 1.5 g administered every 8 h over 1 h.** **(10) For testing and reporting of *H. influenzae* only.**
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	-	-	**(11) See comment (10).** **(12)** Breakpoints are based on a dosage regimen of 600 mg administered every 12 h.
O	Cefonicid	30 µg	≥20	17-19	≤16	≤4	8	≥16	See comment (8).

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	
TETRACYCLINES									
(13) Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. However, resistance to doxycycline and minocycline cannot be inferred from tetracycline resistance.									
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	-	-	

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). **(14)** Not routinely reported on organisms isolated from the urinary tract.
ANSAMYCINS									
C	Rifampin	5 µg	≥20	17-19	≤16	≤1	2	≥4	**(15)** May be appropriate only for prophylaxis of case contacts. These breakpoints do not apply to therapy of patients with invasive *H. influenzae* disease.
PLEUROMUTILINS									
C	**Lefamulin**	**20 µg**	**≥ 17**	**-**	**-**	**≤2**	**-**	**-**	**(16) The breakpoints for susceptible are based on a dosage regimen of 150 mg IV or 600 mg orally administered every 12 h.** **See comments (10) and (14).**

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

Footnote

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

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. 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®[a] 49226

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

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 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 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.

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

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	Cefepime	30 µg	≥31	-	-	≤0.5	-	-	
O	Cefotaxime	30 µg	≥31	-	-	≤0.5	-	-	
O	Cefotetan	30 µg	≥26	20-25	≤19	≤2	4	≥8	See general comment (2).
O	Ceftizoxime	30 µg	≥38	-	-	≤0.5	-	-	
CEPHEMS (ORAL)									
A	Cefixime	5 µg	≥ 31	-	-	≤ 0.25	-	-	
O	Cefpodoxime	10 µg	≥ 29	-	-	≤0.5	-	-	

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	
MACROLIDES									
A	Azithromycin	**15 µg**	**≥ 30**	-	-	≤ 1	-	-	**(8)** This breakpoint presumes that azithromycin (1 g single dose) is used in an approved regimen that includes an additional antimicrobial agent (ie, ceftriaxone 250 mg IM single dose).
TETRACYCLINES									
(9) 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	**(10)** 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	
AMINOCYCLITOLS									
O	Spectinomycin	100 µg	≥ 18	15-17	≤ 14	≤ 32	64	≥ 128	See general comment (2).

Abbreviations: ATCC®, American Type Culture Collection; I, intermediate; IM, intramuscular; MHB, Mueller-Hinton broth; MIC, minimal inhibitory concentration; NAD, ß-nicotinamide adenine dinucleotide; pH, negative logarithm of hydrogen ion concentration; QC, quality control; R, resistant; S, susceptible.

Footnote

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

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

Testing Conditions

Medium: Disk diffusion: MHA with 5% sheep blood or MH-F agar (MHA with 5% defibrinated horse blood and 20 µg/mL NAD)
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®[a] 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.

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 (see the *M02 Disk Diffusion Reading Guide*[2]). 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 compared with 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. The *in vitro* activity of these agents 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.

(5) For disk diffusion, results using MHA with 5% sheep blood and MH-F agar were equivalent when disk contents, testing conditions, and zone diameter breakpoints in Table 2G were used. Disk diffusion QC ranges for *S. pneumoniae* ATCC® 49619 in Table 4B apply to testing using either MHA with 5% sheep blood or MH-F agar.

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									
6) For nonmeningitis isolates, a penicillin MIC of ≤ 0.06 µg/mL (or oxacillin zone ≥ 20 mm) can predict susceptibility to the following ß-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	-	-	-	-	-	**(7)** Isolates of pneumococci with oxacillin zone sizes ≥ 20 mm are susceptible (MIC ≤ 0.06 µg/mL) to penicillin. Penicillin and cefotaxime, ceftriaxone, or meropenem MICs should be determined for isolates with oxacillin zone diameters ≤ 19 mm, because zones ≤ 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	**(8)** ***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. **(9)** For all isolates other than those from CSF, report interpretations for both meningitis and nonmeningitis.
A	Penicillin parenteral (meningitis)	-	-	-	-	≤ 0.06	-	≥ 0.12	**(10)** ***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). **(11)** For CSF isolates, report only meningitis interpretations. See general comment (4).

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)									
A	Penicillin (oral penicillin V)	-	-	-	-	≤0.06	0.12-1	≥2	**(12)** Interpretations for oral penicillin may be reported for isolates other than those from CSF.
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 (6).									
O	Cefepime (meningitis)	-	-	-	-	≤0.5	1	≥2	**(13)** 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	**(14)** In the United States, report only interpretations for nonmeningitis and include the nonmeningitis notation on the report.
B	Cefotaxime (meningitis)	-	-	-	-	≤0.5	1	≥2	**(15)** For CSF isolates, report only meningitis interpretations. **(16)** ***Rx:*** Use of cefotaxime or ceftriaxone in meningitis requires therapy with maximum doses. See general comment (4).
B	Ceftriaxone (meningitis)	-	-	-	-	≤0.5	1	≥2	

Table 2G. *Streptococcus pneumoniae* (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm S	I	R	Interpretive Categories and MIC Breakpoints, µg/mL S	I	R	Comments
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.) (Continued)									
B B	Cefotaxime (nonmeningitis) Ceftriaxone (nonmeningitis)	- -	- -	- -	- -	≤1 ≤1	2 2	≥4 ≥4	**(17)** For all isolates other than those from CSF, report interpretations for both meningitis and nonmeningitis.
C	Ceftaroline (nonmeningitis)	30 µg	≥26	-	-	≤0.5	-	-	**(18)** Breakpoints are based on a dosage regimen of 600 mg administered every 12 h.
C	Cefuroxime (parenteral)	-	-	-	-	≤0.5	1	≥2	
CEPHEMS (ORAL)									
See comment (6).									
C	Cefuroxime (oral)	-	-	-	-	≤1	2	≥4	**(19)** Interpretations for oral cefuroxime may be reported for isolates other than those from CSF.
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	
CARBAPENEMS									
See comment (6).									
B	Meropenem	-	-	-	-	≤0.25	0.5	≥1	See general comment (4) and comment (7).
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									
(20) Susceptibility and resistance to azithromycin, clarithromycin, and dirithromycin can be predicted by testing erythromycin. **(21)** 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	
TETRACYCLINES									
(22) Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline. However, resistance to doxycycline cannot be inferred from tetracycline resistance.									
B B	Tetracycline Doxycycline	30 µg 30 µg	≥28 ≥28	25-27 25-27	≤24 ≤24	≤1 ≤0.25	2 0.5	≥4 ≥1	

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	
FLUOROQUINOLONES									
B B B	Gemifloxacin Levofloxacin Moxifloxacin	5 µg 5 µg 5 µg	≥23 ≥17 ≥18	20-22 14-16 15-17	≤19 ≤13 ≤14	≤0.12 ≤2 ≤1	0.25 4 2	≥0.5 ≥8 ≥4	**(23)** *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.
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 (21).
ANSAMYCINS									
C	Rifampin	5 µg	≥19	17-18	≤16	≤1	2	≥4	**(24)** ***Rx:*** Rifampin should not be used alone for antimicrobial therapy.
LINCOSAMIDES									
B	Clindamycin	2 µg	≥19	16-18	≤15	≤0.25	0.5	≥1	**(25)** For isolates that test erythromycin resistant and clindamycin susceptible or intermediate, testing for ICR by disk diffusion using the D-zone test or by broth microdilution is required before reporting clindamycin (see Table **3I**, Subchapter 3.9 in M02,[3] and Subchapter 3.12 in M07[1]). See comment (21).
STREPTOGRAMINS									
O	Quinupristin-dalfopristin	15 µg	≥19	16-18	≤15	≤1	2	≥4	
OXAZOLIDINONES									
C	Linezolid	30 µg	≥21	-	-	≤2	-	-	
PLEUROMUTILINS									
B	**Lefamulin**	**20 µg**	**≥17**	-	-	**≤0.5**	-	-	**(26) The susceptible breakpoints are based on a dosage regimen of 150 mg IV or 600 mg orally administered every 12 h.** **(27) Not routinely reported on organisms isolated from the urinary tract.**

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; CSF, cerebrospinal fluid; FDA, US Food and Drug Administration; I, intermediate; ICR, inducible clindamycin resistance; IV, intravenous; LHB, lysed horse blood; MHA, Mueller-Hinton agar; MH-F agar, Mueller-Hinton fastidious agar; MIC, minimal inhibitory concentration; NAD, β-nicotinamide adenine dinucleotide; QC, quality control; R, resistant; S, susceptible.

Table 2G. *Streptococcus pneumoniae* (Continued)

Footnote

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

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

References for Table 2G

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

2 CLSI. *M02 Disk Diffusion Reading Guide*. 1st ed. CLSI quick guide M02QG. Clinical and Laboratory Standards Institute; 2018.

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

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®[a] 49619

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

Refer to Table 3I 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 (see the *M02 Disk Diffusion Reading Guide*[2]). 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 *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.

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

(5) Breakpoints for *Streptococcus* spp. ß-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 ß-hemolytic streptococci, penicillin is **tested as** a surrogate for ampicillin, amoxicillin, amoxicillin-clavulanate, ampicillin-sulbactam, cefazolin, cefepime, ceftaroline, cephradine, cephalothin, cefotaxime, ceftriaxone, ceftizoxime, imipenem, ertapenem, and meropenem. For group A ß-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 administered 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*, and *S. dysgalactiae*.
C	Oritavancin	-	-	-	-	≤0.25	-	-	
C	Telavancin	-	-	-	-	≤0.12	-	-	
LIPOPEPTIDES									
C	Daptomycin	-	-	-	-	≤1	-	-	**(9)** Daptomycin should not be reported for isolates from the respiratory tract.

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 ICR) should be tested, and only clindamycin should be reported. Erythromycin should be tested for ICR determination only and should not be reported. See Table **3I.**
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. However, resistance to doxycycline and minocycline cannot be inferred from tetracycline resistance.									
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).

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	
LINCOSAMIDES									
A	Clindamycin	2 µg	≥19	16-18	≤15	≤0.25	0.5	≥1	See comments (11) and (12). **(14)** For isolates that test erythromycin resistant and clindamycin susceptible or intermediate, testing for ICR by disk diffusion using the D-zone test or by broth microdilution is required before reporting clindamycin. See Table **3I,** Subchapter 3.9 in M02,[3] and Subchapter 3.12 in M07.[1]
STREPTOGRAMINS									
O	Quinupristin-dalfopristin	15 µg	≥19	16-18	≤15	≤1	2	≥4	**(15) For reporting** against *S. pyogenes* **only.**
OXAZOLIDINONES									
(16) ***S. agalactiae*** **and** ***S. pyogenes*** **that test susceptible to linezolid by MIC are also considered susceptible to tedizolid. However, some organisms that are nonsusceptible to linezolid may be susceptible to tedizolid.**									
C	Linezolid	30 µg	≥21	-	-	≤2	-	-	
C	Tedizolid	-	-	-	-	≤0.5	-	-	**(17)** For reporting against *S. pyogenes* and *S. agalactiae* only.

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

Footnote

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

References for Table 2H-1

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

[2] CLSI. *M02 Disk Diffusion Reading Guide*. 1st ed. CLSI quick guide M02QG. Clinical and Laboratory Standards Institute; 2018.

[3] CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.

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®[a] 49619

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

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 ß-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.

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 A	Penicillin Ampicillin	-	-	-	-	≤0.12 ≤0.25	0.25-2 0.5-4	≥4 ≥8	**(5)** Viridans streptococci isolated from normally sterile anatomical 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.
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 administered 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. anginosus* group (includes *S. anginosus*, *S. intermedius*, and *S. constellatus*) only.
C	Oritavancin	-	-	-	-	≤0.25	-	-	
C	Telavancin	-	-	-	-	≤0.06	-	-	
LIPOPEPTIDES									
O	Daptomycin	-	-	-	-	≤1	-	-	**(10)** Daptomycin should not be reported for isolates from the respiratory tract.

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. However, resistance to doxycycline and minocycline cannot be inferred from tetracycline resistance.									
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									
(14) ***S. anginosus*** **group that test susceptible to linezolid by MIC are also considered susceptible to tedizolid. However, some organisms that are nonsusceptible to linezolid may be susceptible to tedizolid.**									
C	Linezolid	30 µg	≥21	-	-	≤2	-	-	
C	Tedizolid	-	-	-	-	≤0.25	-	-	See comment (9).

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.

Footnote

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

Reference for Table 2H-2

1 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. 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 considered 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®[a] 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.

General Comments

Important: For complete information on safety precautions, see *Biosafety in Microbiological and Biomedical Laboratories*. 6th ed. Washington, DC: US Department of Health and Human Services; 2020. http://www.cdc.gov/biosafety/publications/bmbl5/. Accessed 1 February 2021.

(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. http://www.cdc.gov/vaccines/acip/index.html. Accessed 15 February 2021.

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.

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									
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	

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.

Footnote

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

Reference for Table 2I

1 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. 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 *Bacteroides* spp. and *Parabacteroides* spp. 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®[a] 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.

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 recommended only for testing *Bacteroides* spp. and *Parabacteroides* spp. MIC values for agar or broth microdilution are considered equivalent for those species.

(5) Until additional studies are performed to validate broth microdilution for testing other organisms, it should be used only for testing members of *Bacteroides* spp. and *Parabacteroides* spp.

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

Table 2J. Anaerobes (Continued)

Test/Report Group	Antimicrobial Agent	Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	
PENICILLINS					
A/C A/C	Ampicillin[b] Penicillin[b]	≤0.5 ≤0.5	1 1	≥2 ≥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)** *Bacteroides* spp. are intrinsically resistant to penicillin and ampicillin. *Parabacteroides* spp. are presumed to be resistant to penicillin and ampicillin. 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.
β-LACTAM COMBINATION AGENTS					
A A A	Amoxicillin-clavulanate Ampicillin-sulbactam Piperacillin-tazobactam	≤4/2 ≤8/4 ≤16/4	8/4 16/8 32/4-64/4	≥16/8 ≥32/16 ≥128/4	
B	**Imipenem-relebactam**	**≤4/4**	**8/4**	**≥16/4**	**(9) Breakpoints are based on a dosage regimen of 1.25 g administered every 6 h.** **(10) Organisms that test susceptible to imipenem are also considered susceptible to imipenem-relebactam. However, organisms that test susceptible to imipenem-relebactam cannot be assumed to be susceptible to imipenem.**
O	Ticarcillin-clavulanate	≤32/2	64/2	≥128/2	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)					
C C	Cefotetan Cefoxitin	≤16 ≤16	32 32	≥64 ≥64	
C C	Ceftizoxime Ceftriaxone	≤32 ≤16	64 32	≥128 ≥64	
O	Cefmetazole	≤16	32	≥64	
O	Cefoperazone	≤16	32	≥64	
O	Cefotaxime	≤16	32	≥64	

Table 2J. Anaerobes (Continued)

Test/Report Group	Antimicrobial Agent	Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	
CARBAPENEMS					
A	Doripenem	≤2	4	≥8	
A	Ertapenem	≤4	8	≥16	
A	Imipenem	≤4	8	≥16	**See comment (10).**
A	Meropenem	≤4	8	≥16	
TETRACYCLINES					
C	Tetracycline	≤4	8	≥16	
FLUOROQUINOLONES					
C	Moxifloxacin	≤2	4	≥8	
LINCOSAMIDES					
A	Clindamycin	≤2	4	≥8	
PHENICOLS					
C	Chloramphenicol	≤8	16	≥32	
NITROIMIDAZOLES					
A	Metronidazole	≤8	16	≥32	**(11)** 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.

Footnotes

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

b. A/C: Group A for gram-positive anaerobes and group C for gram-negative organisms. Refer to Table 1C.

Reference for Table 2J

1 CLSI. *Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria.* 9th ed. CLSI standard M11. Clinical and Laboratory Standards Institute; 2018.

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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 prevention purposes. For laboratories that have not implemented the current breakpoints, ESBL testing should be performed as described in this table.

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 pneumoniae*, *Klebsiella oxytoca*, or *Proteus mirabilis*, 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 (Testing 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 (Testing 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

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 2-fold 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, test interpretations for these agents do not need to be changed from susceptible to resistant.	

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).	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).	When performing the ESBL test, *K. pneumoniae* ATCC® 700603 and *E. coli* ATCC® 25922 should be used for routine QC (eg, weekly or daily).	When performing the ESBL test, *K. pneumoniae* ATCC® 700603 and *E. coli* ATCC® 25922 should be tested routinely (eg, weekly or daily).
	E. coli ATCC® 25922 (see acceptable QC ranges in Table 4A-1)	*E. coli* ATCC® 25922 = no growth (see acceptable QC ranges listed in Table 5A-1)	**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.	**Acceptable QC:** *E. coli* ATCC® 25922: < 3 2-fold 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: Cefpodoxime zone 9-16 mm Ceftazidime zone 10-18 mm Aztreonam zone 10-16 mm Cefotaxime zone 17-25 mm Ceftriaxone zone 16-24 mm	*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	*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.	*K. pneumoniae* ATCC® 700603: ≥ 3 2-fold 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.

Table 3A. (Continued)

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.

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

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

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

Carbapenemase-producing isolates of Enterobacterales usually test intermediate or resistant to one or more carbapenems using the current breakpoints as listed in Table 2A (**NOTE:** Testing not susceptible to ertapenem is often 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 Enterobacterales MIC breakpoints for carbapenems described in M100-S20 (January 2010) should perform the CarbaNP test, mCIM, eCIM, and/or a molecular assay (refer to Tables 3B and 3C for methods) when isolates of Enterobacterales are suspicious for carbapenemase production based on imipenem or meropenem MICs 2–4 µg/mL or ertapenem MIC 2 µg/mL (refer to Tables 3B-1 and 3C-1 for guidance on reporting). After implementing the current breakpoints, these additional tests may not need to be performed other than for epidemiological or infection prevention purposes (ie, it is no longer necessary to edit results for the carbapenems to resistant if a carbapenemase producer is detected).

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

	Tests Used for Epidemiological or Infection Prevention-Related Testing			
	CarbaNP (Table 3B)	mCIM (Table 3C)	mCIM With eCIM (Table 3C)	Other (eg, molecular assays)
Organisms	Enterobacterales and *P. aeruginosa* that are not susceptible to one or more carbapenems	Enterobacterales and *P. aeruginosa* that are not susceptible to one or more carbapenems	Enterobacterales that are positive by mCIM	Enterobacterales 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.

Table 3B. CarbaNP Test for Suspected Carbapenemase Production in Enterobacterales 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 3B-1.

Test	CarbaNP Test
When to perform this test	For epidemiological or infection prevention 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.

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

Table 3B. (Continued)

Test	CarbaNP Test
Test interpretation (Continued)	**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; pH, negative logarithm of hydrogen ion concentration; QC, quality control.

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.

NOTE 1: Test recommendations were largely derived following testing of US isolates of Enterobacterales and *P. aeruginosa* and provide for a high level of sensitivity (> 90%) and specificity (> 90%) in detecting KPC, NDM, VIM, IMP, SPM, and SME-type carbapenemases in these isolates. The sensitivity and specificity of the test for detecting other carbapenemase production can vary. The ability of this test, as listed in the above procedure, to detect OXA-48-like producers is poor.[6,7]

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.

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 perform 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 Enterobacterales 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). 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 Enterobacterales are CarbaNP positive.

Abbreviation: MIC, minimal inhibitory concentration.

Tables 3B and 3B-1. Instructions for Preparing 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.

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

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

Step	Action	Comment
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	Comment
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.	

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

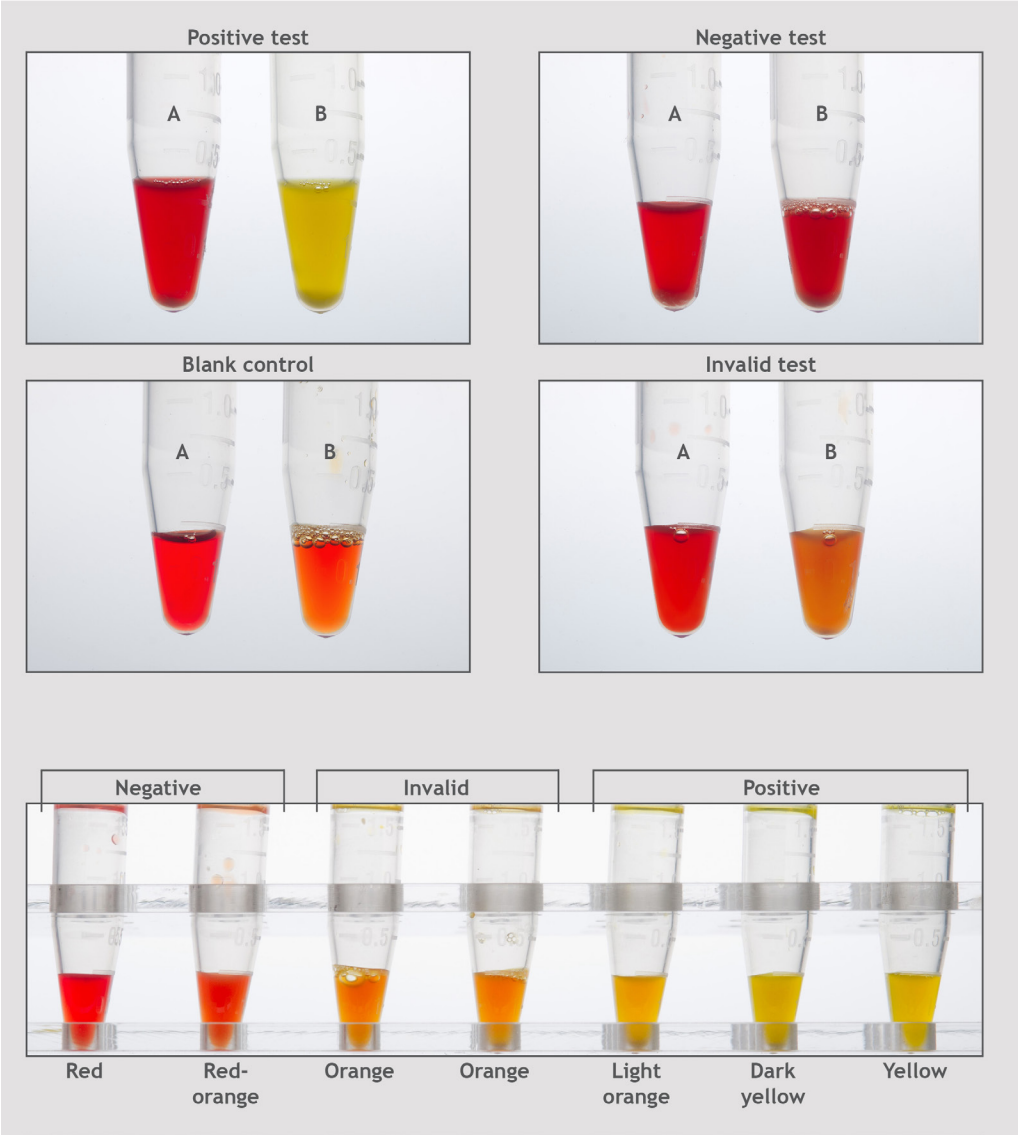


Figure 1. Interpretation of Color Reactions

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.

6 Lutgring JD, Zhu W, de Man TJB, et al. Phenotypic and genotypic characterization of Enterobacteriaceae producing oxacillinase-48-like carbapenemases, United States. *Emerg Infect Dis*. 2018;24(4):700-709.

7 Cunningham SA, Limbago B, Traczewski M, et al. Multicenter performance assessment of Carba NP test. *J Clin Microbiol*. 2017;55(6):1954-1960.

Table 3C. Modified Carbapenem Inactivation Methods for Suspected Carbapenemase Production in Enterobacterales and *Pseudomonas aeruginosa*[1-6]

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

Test	mCIM Only or in Conjunction With eCIM
When to perform this test:	For epidemiological or infection prevention 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 Enterobacterales and *P. aeruginosa* whereas eCIM is used together with mCIM to differentiate metallo-ß-lactamases from serine carbapenemases in Enterobacterales. • mCIM can be performed alone; however, eCIM must be performed together with mCIM. • eCIM is valid only 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)

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 Enterobacterales 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 Enterobacterales 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).

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-ß-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-ß-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 compared with the mCIM zone diameter. • Metallo-ß-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 with the mCIM zone diameter.

Table 3C. (Continued)

Test	mCIM Only or in Conjunction With eCIM
Reporting	**mCIM Only**

mCIM Result	eCIM Result	Report
Negative	Not set up	Carbapenemase not detected
Positive	Not set up	Carbapenemase detected
Indeterminate	Not set up	Testing inconclusive for the presence of carbapenemase. Call laboratory to discuss.*

mCIM and eCIM Combination Test

mCIM Result	eCIM Result	Report
Negative	Do not interpret	Carbapenemase not detected
Positive	Negative	Serine carbapenemase detected
Positive	Positive	Metallo-ß-lactamase detected
Indeterminate	Do not interpret	Testing inconclusive for the presence of carbapenemase. Call laboratory to discuss.[a]

[a] 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-ß-lactamase are co-produced by one organism, differentiation between enzymes will not be possible and false-negative eCIM results may occur.

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 Enterobacterales 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™[a] / NDM positive, Metallo-ß-lactamase producer / mCIM positive, eCIM positive [a] 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; mCIM, modified carbapenem inactivation method; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; TSB, trypticase soy broth.

Table 3C. (Continued)

Footnotes

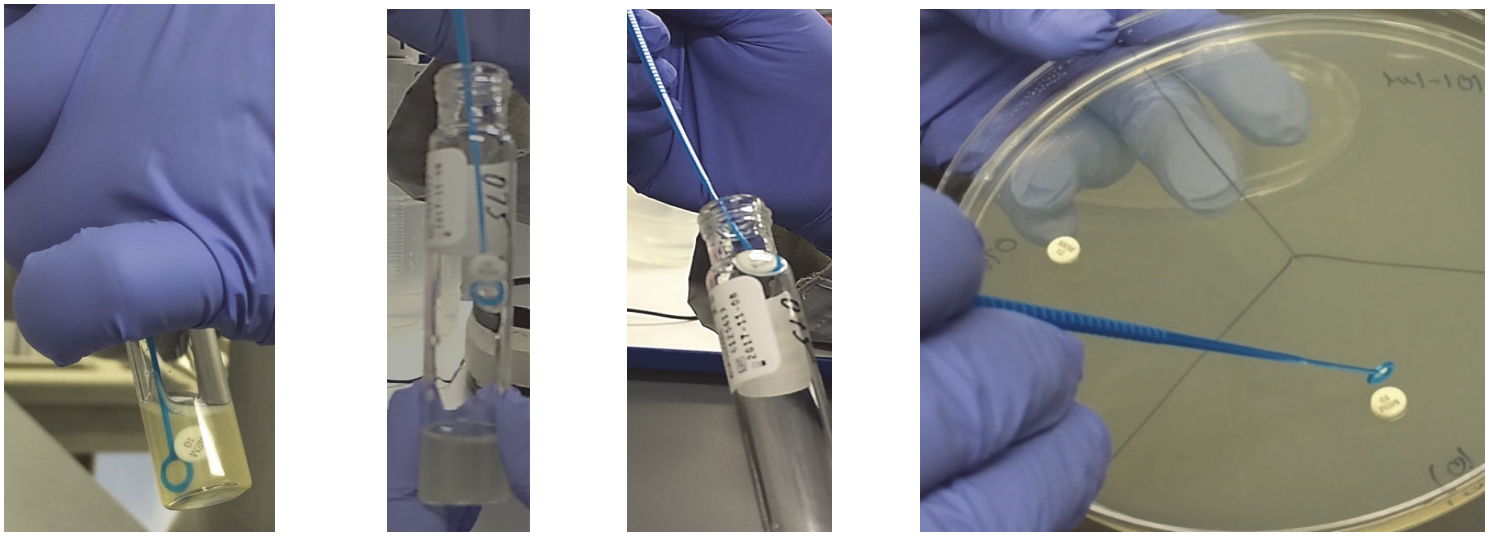
a. ATCC® is a registered trademark of the American Type Culture Collection. Per ATCC® convention, the trademark symbol is used after "BAA" in each catalog number, in conjunction with the registered ATCC® name.

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 and Prevention in collaboration with the US Food and Drug Administration.

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 Enterobacterales 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-Enterobacterales 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 Enterobacterales isolates investigated by CLSI.[b] In CLSI studies, one *K. pneumoniae* co-producing NDM and OXA-181 yielded a false-negative result at 3 of 4 validation sites.

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.

Table 3C. (Continued)

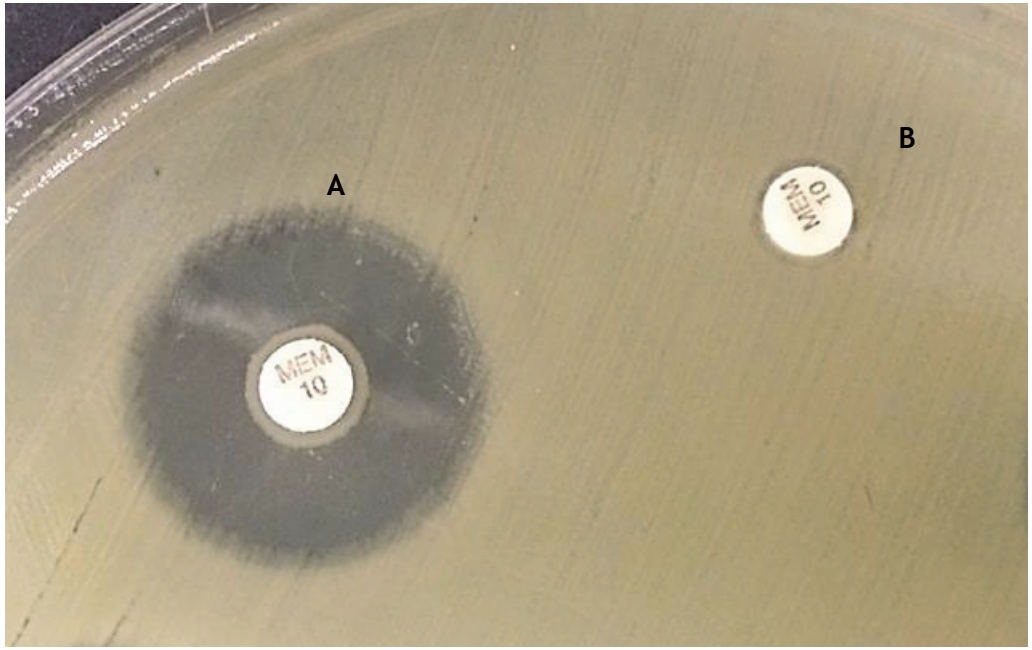


Figure 2A. mCIM Results for QC Strains: Negative Control *K. pneumoniae* ATCC® BAA-1706™ (A) and Positive Control *K. pneumoniae* ATCC® BAA-1705™ (B). **NOTE:** A narrow ring of growth around the meropenem disk as seen with the negative control (A) results from carryover of the test organism in the TSB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

Table 3C. (Continued)

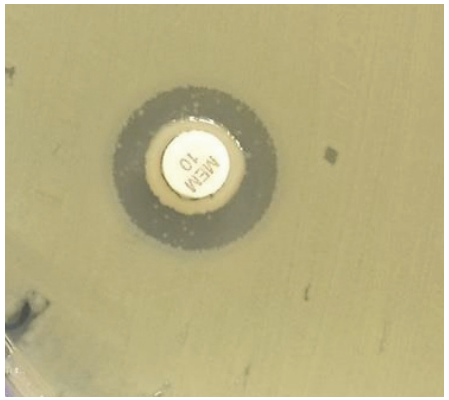


Figure 2B. mCIM Test Interpretation

- 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.

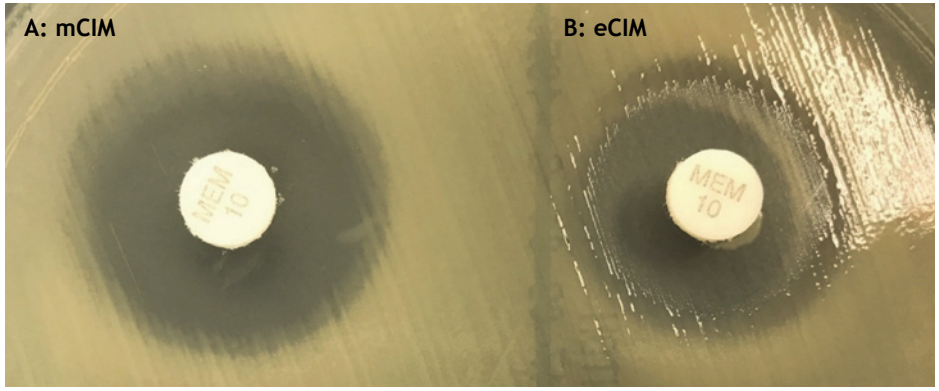


Figure 3A. mCIM and eCIM Test Interpretation: Negative mCIM. "A" shows an mCIM negative result (zone diameter = 20 mm) and "B" shows an eCIM invalid result. Do not interpret the eCIM result when the mCIM is negative as the isolate is negative for carbapenemase production.

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

Table 3C. (Continued)

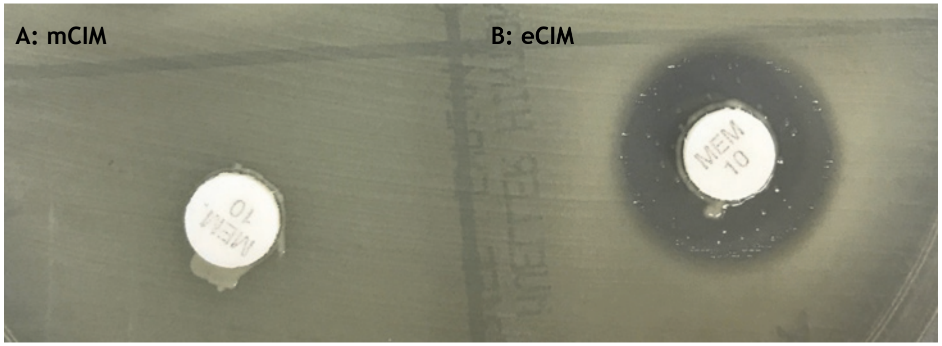


Figure 3B. mCIM and eCIM Test Interpretation: Positive mCIM and eCIM. "A" shows an mCIM positive result (zone diameter of 6 mm) and "B" shows an eCIM positive result (zone diameter = 15 mm with pinpoint colonies throughout the zone of inhibition). **NOTE:** The pinpoint colonies throughout the zone of 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

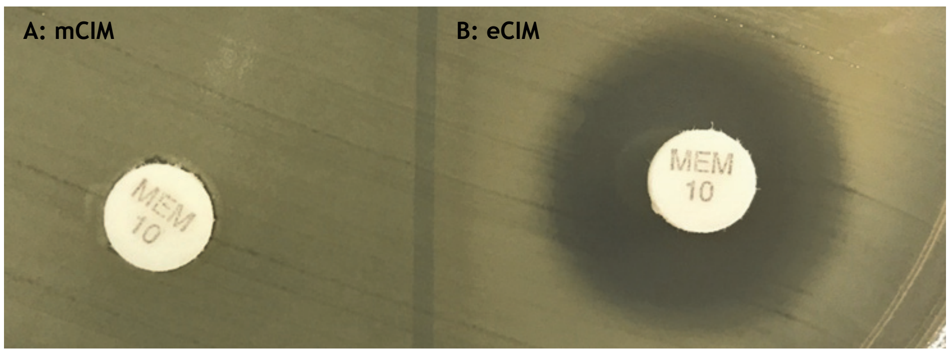


Figure 3C. mCIM and eCIM Test Interpretation: Positive mCIM and eCIM. "A" shows an mCIM positive result (zone diameter = 6 mm) and "B" shows an eCIM positive result (zone diameter = 19 mm). A ≥ 5-mm increase in zone diameter for eCIM vs diameter for mCIM zone (19 mm – 6 mm = 13 mm) demonstrates the inhibition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

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

Table 3C. (Continued)

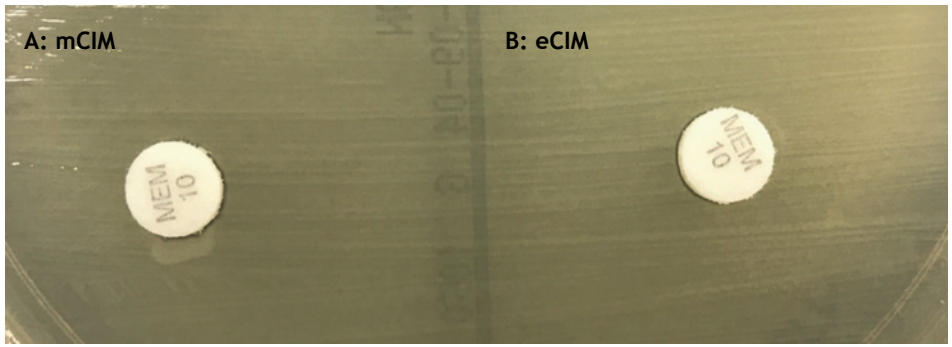


Figure 3D. mCIM and eCIM Test Interpretation: Positive mCIM and Negative eCIM. "A" shows an mCIM positive result (zone diameter = 6 mm) and "B" shows an eCIM negative result (zone diameter = 6 mm). Serine carbapenemases are not inhibited by EDTA and demonstrate a ≤ 4-mm increase in zone diameter for 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. Clinical and Laboratory Standards Institute; 2018.

5 Simner PJ, Johnson JK, Brasso WB, et al. Multicenter evaluation of the modified carbapenem inactivation method and the Carba NP for detection of carbapenemase-producing *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. *J Clin Microbiol*. 2017;56(1):e01369-17.

6 Sfeir MM, Hayden JA, Fauntleroy KA, et al. EDTA-modified carbapenem inactivation method: a phenotypic method for detecting metallo-ß-lactamase-producing Enterobacteriaceae. *J Clin Microbiol*. 2019;57(5):e01757-18.

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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 perform 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 Enterobacterales 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). 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 Enterobacterales are mCIM positive.

Abbreviations: mCIM, modified carbapenem inactivation method; MIC, minimal inhibitory concentration.

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Table 3D. Tests for Colistin Resistance for Enterobacterales and *Pseudomonas aeruginosa*

The polymyxins (colistin and polymyxin B) are antimicrobial agents of last resort for treating multidrug-resistant infections. Clinical and PK/PD data suggest that these agents have limited clinical efficacy. Alternative agents are strongly preferred. If these agents are not available, knowledge of the colistin MIC may be helpful to inform treatment decisions.

For colistin, broth microdilution, broth disk elution and agar dilution MIC methods are acceptable. Broth microdilution is the only approved method for polymyxin B. Disk diffusion and gradient diffusion methods should not be performed.

Colistin and polymyxin B are considered equivalent agents, so MICs obtained from testing colistin predict MICs to polymyxin B and vice versa. At this time, CLSI has not evaluated polymyxin B testing methods, and the procedures below should not be adapted to polymyxin B. The methods below were evaluated for *Acinetobacter* spp. by CLSI and found to yield inaccurate results.

These methods were established with limited disk and/or media manufacturers and are considered provisional until additional data are evaluated by CLSI and shown to meet CLSI document M23[1] guidelines.

Test	Colistin Broth Disk Elution	Colistin Agar Test
Approved organisms	Enterobacterales and *Pseudomonas aeruginosa*	Enterobacterales and *P. aeruginosa*
Strengths	No special reagents or media necessary	Ability to test up to 10 isolates at one time
Limitations	Hands-on time and cost	Requires special media (colistin agar plate)
When to perform this test	Testing multidrug-resistant isolates for clinical or infection prevention purposes	Testing multidrug-resistant isolates for clinical or infection prevention purposes
Test method	Tube dilution using colistin disk as the colistin source	Agar dilution: slight variation of method described in M07[2] (ie, different inoculum and different approach to interpreting results)
Organism group	Enterobacterales and *P. aeruginosa*	Enterobacterales and *P. aeruginosa*
Medium	CAMHB (10-mL tubes)	MHA (20 mL in 100-mm Petri plate)[a]
Antimicrobial concentration	10-µg colistin **sulfate** disks Final concentration: 0 µg/mL (growth control), 1 µg/mL, 2 µg/mL, and 4 µg/mL colistin	**Colistin sulfate** Final concentration: 0 µg/mL (growth control), 1 µg/mL, 2 µg/mL, and 4 µg/mL colistin[a]
Inoculum	1. Using a loop or swab, pick 3-5 colonies from a fresh (18-24 hours) nonselective agar plate and transfer to sterile saline (4-5 mL). 2. Adjust turbidity to equivalent of a 0.5 McFarland turbidity standard.	1. Using a loop or swab, pick 3-5 colonies from a fresh (18-24 hours) nonselective agar plate and transfer to sterile saline (4-5 mL). 2. Adjust turbidity to equivalent of a 0.5 McFarland turbidity standard. 3. Dilute the standardized inoculum 1:10 in saline.

Table 3D. (Continued)

Test	Colistin Broth Disk Elution	Colistin Agar Test
Test procedure	1. Let the CAMHB tubes (10 mL) and colistin disks warm to room temperature. 2. Label 4 tubes of CAMHB for each isolate to be tested with 1, 2, and 4 µg/mL and control (see Figure 1). 3. Using aseptic technique, carefully add: • 1 colistin disk to the tube labeled "1 µg/mL" • 2 colistin disks to tube labeled "2 µg/mL" • 4 colistin disks to the tube labeled "4 µg/mL" 4. Gently vortex the tubes with the added disk and let the colistin elute from the disks for at least 30 minutes but no longer than 60 minutes at room temperature. 5. Prepare the standardized inoculum. 6. Add 50 µL standardized inoculum to the control and 1-, 2-, and 4-µg/mL tubes to attain a final inoculum concentration of approximately 7.5×10^5 CFU/mL. 7. Using a 10-µL loop, subculture from the original inoculum tube to a blood agar plate as a purity check. 8. Cap the tubes tightly and vortex each inoculated tube on slow speed to mix. Slow speed is suggested to prevent colistin from sticking to the cap and glass surface above the meniscus of liquid. 9. Loosen the caps slightly before incubation. 10. Incubate the tubes and purity plate.	1. Divide each colistin agar plate with increasingly doubled dilutions of colistin in up to 10 parts, with a marker to test up to 10 isolates per plate. Label each part with the appropriate isolate number (see Figure 2). 2. Using a pipette or a 10-µL loop, streak 10 µL of the 1:10 dilution onto the appropriate part of each colistin agar plate. 3. Using a 10-µL loop, subculture from the original inoculum tube to a blood agar plate as a purity check. 4. Incubate the colistin agar plates and purity plate.
Incubation conditions	33 to 35°C; ambient air	33 to 35°C; ambient air
Incubation length	16–20 hours	16–20 hours

Table 3D. (Continued)

Test	Colistin Broth Disk Elution	Colistin Agar Test
Results	1. Examine the purity plate to ensure inoculum was pure. 2. Examine the growth control tube, which must demonstrate obvious turbidity for the test to be valid. NOTE: Some *P. aeruginosa* isolates may grow only near the meniscus. 3. Read the MIC as the lowest concentration that completely inhibits growth of the test isolate. (See Figure 1 for examples.) For Enterobacterales and *P. aeruginosa:* • ≤ 2 µg/mL = intermediate • ≥ 4 µg/mL = resistant	1. Examine the purity plate to ensure inoculum was pure. 2. Examine the growth control plate, which must demonstrate confluent growth for the test to be valid. 3. Examine the colistin plates carefully with transmitted light for colony or light film of growth. 4. Read the MIC as the lowest colistin agar plate concentration that completely inhibits growth of the test isolate (eg, even 1 colony would be considered growth). See Figure 2 for examples. For Enterobacterales and *P. aeruginosa:* • ≤ 2 µg/mL = intermediate • ≥ 4 µg/mL = resistant
Additional testing and reporting	If there is an inconsistent growth pattern (eg, no growth in 2 µg/mL but growth at 1 µg/mL and 4 µg/mL), repeat the test. An inconsistent growth pattern may occur as a result of: • Contamination at higher dilutions • Heteroresistance • Improper concentrations of antimicrobial agent in the tubes • Error inoculating the tubes	If there is an inconsistent growth pattern (eg, no growth in 2 µg/mL but growth at 1 µg/mL and 4 µg/mL), repeat the test. An inconsistent growth pattern may occur as a result of: • Contamination at higher dilutions • Heteroresistance • Improper concentrations of antimicrobial agent in the colistin agar plates • Error inoculating the plates
QC recommendations - routine[b]	*Escherichia coli* AR Bank #0349 *mcr-1* (≤ 1-4 µg/mL, with a target of 2 µg/mL)[c] and *P. aeruginosa* ATCC®[d] 27853 (≤ 1-4 µg/mL)	*E. coli* AR Bank #0349 *mcr-1* (≤ 1-4 µg/mL, with a target of 2 µg/mL)[c] and *P. aeruginosa* ATCC® 27853 (≤ 1-4 µg/mL)

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; CFU, colony-forming unit(s); MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; PK/PD, pharmacokinetic/pharmacodynamic; QC, quality control.

Table 3D. (Continued)

Footnotes

a. Refer to M07[2] for preparation of media and antimicrobial agents.

b. QC recommendations – routine

Test recommended routine QC strains:

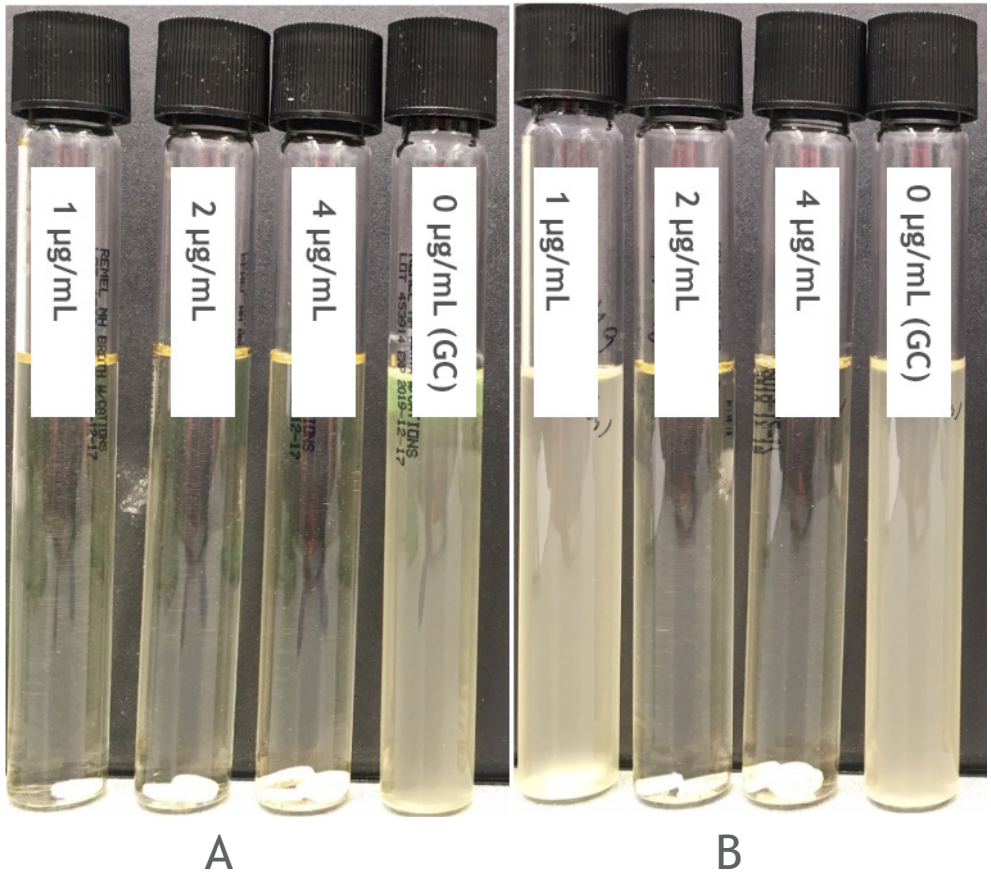
- Weekly if the test is performed at least once a week and criteria for converting from daily to weekly QC testing have been met (see Subchapter 4.7.2.3 in M02[3] and M07[2]) and the individualized QC plan is complete
- Daily if the test is performed less than once per week and/or if criteria for converting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

Perform QC of colistin disks and test media daily or weekly following the routine disk diffusion QC procedure and handle disks as described in M02.[3]

c. The QC ranges were established with disks (colistin broth disk elution) and media from a limited number of manufacturers and are considered provisional until additional data are evaluated by CLSI and shown to meet CLSI document M23[1] guidelines.

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

Table 3D. (Continued)



Abbreviation: GC, growth control.

Figure 1. Colistin Broth Disk Elution. Results for routine QC strain *P. aeruginosa* ATCC® 27853 with an MIC ≤ 1 µg/mL (A) and supplemental QC strain *E. coli* AR Bank #0349 *mcr-1* with an MIC 2 µg/mL (B).

Table 3D. (Continued)

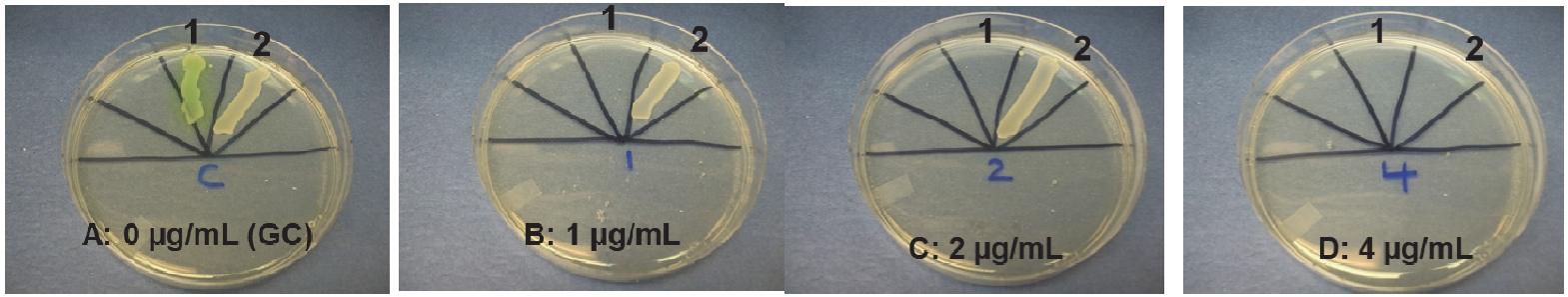


Figure 2. Colistin Agar Test. The plates need to be examined carefully with transmitted light for confluent growth, individual colonies, or light film of growth to determine the MIC. Colistin agar test results for routine QC strain *P. aeruginosa* ATCC® 27853 (position 1) with an MIC ≤ 1 µg/mL and for supplemental QC strain *E. coli* AR Bank #0349 *mcr-1* (position 2) with an MIC 4 µg/mL. The plates shown contain 0 µg/mL (control) (A), 1 µg/mL (B), 2 µg/mL (C), and 4 µg/mL (D) colistin.

References for Table 3D

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

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

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

Table 3E. Test for Performing Disk Diffusion Directly From Positive Blood Culture Broth

Test	Direct Disk Diffusion
Test method	Disk diffusion using positive blood culture broth
Organism group	Enterobacterales
Medium	MHA
Antimicrobial concentration	Standard disk content for the antimicrobials listed below: • Ampicillin 10 µg • Aztreonam 30 µg • Ceftazidime 30 µg • Ceftriaxone 30 µg • Tobramycin 10 µg • Trimethoprim-sulfamethoxazole 1.25/23.75 µg
Inoculum	Positive blood culture broth with gram-negative bacilli, used within 8 hours of flagging positive by the blood culture system
Test procedure	1. Invert blood culture bottle 5-10 times to thoroughly mix. 2. Sterilize the top of the bottle with an alcohol wipe (allow to dry) and insert 20-gauge venting needle into the blood culture bottle. 3. Dispense 4 drops of blood culture broth onto an MHA plate. As a purity check, use an inoculated blood agar plate streaked for isolation. 4. Spread blood culture broth across the entire surface of the MHA plate using a sterile cotton swab. 5. Repeat this procedure by streaking twice more, rotating the plate approximately 60 degrees each time to ensure an even distribution of inoculum. 6. Leave the lid ajar for 3-5 minutes (ideally) but no more than 15 minutes. 7. Dispense antimicrobial disks onto the surface of the inoculated MHA plate. 8. Press each disk down to ensure complete contact with the agar surface. 9. Invert the plate and place in the incubator within 15 minutes of disks being applied.
Incubation conditions	35°C ± 2°C; ambient air
Incubation length	16-18 hours
Results	1. Examine the blood agar purity plate to ensure pure growth. 2. Examine the test plate to ensure confluent lawn of growth appropriate to read disk zone tests per M02.[1] 3. Measure the zone diameters according to routine disk diffusion recommendations in M02.[1] 4. Report results using the interpretive categories and zone diameter breakpoints in Table 2A if the gram-negative bacillus tested is confirmed to be an Enterobacterales. If species is identified as another organism, do not interpret or report results.

Table 3E. (Continued)

Test	Direct Disk Diffusion
Additional testing and reporting	• If there is an inconsistent growth pattern on the plate (eg, mixed inoculum, nonconfluent growth, growth is too faint to read), do not interpret or report results from the direct disk diffusion test, and perform standard susceptibility testing from pure colony growth. • Antimicrobial agents to which the organism is intrinsically resistant (see Appendix B) should be reported as resistant, regardless of measured zone size. • If two zones of growth inhibition are observed, measure the inner zone diameter. In case of colonies present within zones, or presence of both inner and outer zones, check the purity plate and, if pure, record the inner zone diameter.
QC recommendations	*E. coli* ATCC® 25922 Perform QC according to standard disk diffusion QC procedures per M02[1] (eg, daily or weekly).

Abbreviations: ATCC, American Type Culture Collection; MHA, Mueller-Hinton agar; QC, quality control.

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

Reference for Table 3E

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

Table 3F. Test for Detection of ß-Lactamase Production in *Staphylococcus* spp.

Test	ß-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]	*Staphylococcus* spp.[a,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") = ß-lactamase positive (see Figure 1 below this table) Fuzzy zone edge ("beach") = ß-lactamase negative (see Figure 2 below this table)	Nitrocefin-based test: conversion from yellow to red/pink = ß-lactamase positive.
Additional testing and reporting	ß-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. ß-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; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; N/A, not applicable; QC, quality control.

Table 3F. (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 methods 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.

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.

Table 3F. (Continued)

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.

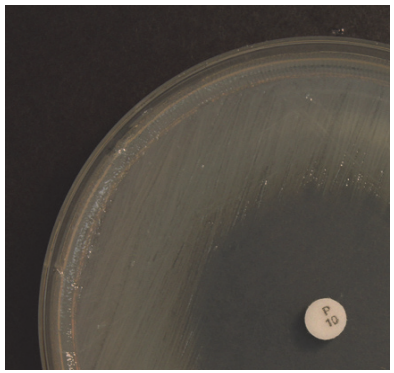


Figure 2. Negative Penicillin Disk Zone-Edge Test for β-Lactamase Detection. The zone edge is fuzzy or like a “beach,” indicating no β-lactamase production.

Table 3F. (Continued)

References for Table 3F

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. Clinical and Laboratory Standards Institute; 2018.

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

Table 3G-1. Test for Detecting Methicillin (Oxacillin) Resistance in *Staphylococcus aureus*[a] and *Staphylococcus lugdunensis*

Test	Detecting *mecA*-Mediated Resistance Using Cefoxitin[b]		Detecting *mecA*-Mediated Resistance Using Oxacillin	Detecting *mecA*-mediated Resistance Using Oxacillin Salt Agar for *S. aureus* Only
Test method	Disk diffusion	Broth microdilution	Broth microdilution and agar dilution	Agar dilution for *S. aureus*
Medium	MHA	CAMHB	CAMHB with 2% NaCl (broth microdilution) MHA with 2% NaCl (agar dilution)	MHA with 4% NaCl
Antimicrobial concentration	30-µg cefoxitin disk	4 µg/mL cefoxitin	2 µg/mL oxacillin	6 µg/mL oxacillin
Inoculum	Standard disk diffusion procedure	Standard broth microdilution procedure	Standard broth microdilution procedure or standard agar dilution procedure	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.
Incubation conditions	33 to 35°C; ambient air[c]			
Incubation length	16–18 hours	16–20 hours	24 hours (may be reported after 18 hours, if resistant)	24 hours; read with transmitted light
Results	≤ 21 mm = positive for *mecA*-mediated resistance ≥ 22 mm = negative for *mecA*-mediated resistance	≥ 8 µg/mL = positive for *mecA*-mediated resistance ≤ 4 µg/mL = negative for *mecA*-mediated resistance	≥4 µg/mL = positive for *mecA*-mediated resistance ≤ 2 µg/mL = negative for *mecA*-mediated resistance	Examine carefully with transmitted light for > 1 colony or light film of growth. > 1 colony = positive for *mecA*-mediated resistance
Additional testing and reporting	Isolates that test positive for *mecA*-mediated resistance should be reported as methicillin (oxacillin) (not cefoxitin) resistant; other β-lactam agents, except ceftaroline, should be reported as resistant or should not be reported.[d]			
QC recommendations - routine[e]	*S. aureus* ATCC®[f] 25923 – *mecA* negative (zone 23–29 mm)	*S. aureus* ATCC® 29213 – *mecA* negative (MIC 1–4 µg/mL)	*S. aureus* ATCC® 29213 – *mecA* negative (MIC 0.12–0.5 µg/mL)	*S. aureus* ATCC®[c] 29213 – susceptible (≤ 1 colony; with each test day)
QC recommendations - lot/shipment[g]	N/A	*S. aureus* ATCC® 43300 – *mecA* positive (MIC ≥ 8 µg/mL)	*S. aureus* ATCC® 43300 – *mecA* positive (MIC ≥ 8 µg/mL)	*S. aureus* ATCC® 43300 – *mecA* positive (>1 colony)

Abbreviations. ATCC, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; MRS, methicillin (oxacillin)-resistant *Staphylococcus* spp.; N/A, not applicable.

Table 3G-1. (Continued)

Footnotes

a. **Including members of the *S. aureus* complex (see Table 2C, comment 2).**

b. Cefoxitin is used as a surrogate test for detecting *mecA*-mediated methicillin (oxacillin) resistance.

c. Testing at temperatures above 35°C may not detect MRS.

d. Testing of other ß-lactam agents, except ceftaroline, is not advised.

e. 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])

f. 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

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

h. 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 3G-1

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

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

Table 3G-2. Test for Detecting Methicillin (Oxacillin) Resistance in *Staphylococcus* spp. Except *Staphylococcus aureus*[a] and *Staphylococcus lugdunensis*

Test	Detecting *mecA*-Mediated Resistance Using Cefoxitin[b]	Detecting *mecA*-Mediated Resistance Using Oxacillin	
Test method	Disk diffusion	Disk diffusion	Broth microdilution and agar dilution
Organism group	*Staphylococcus* spp. except: *S. aureus* (refer to Table 3G-1) *S. lugdunensis* (refer to Table 3G-1) *S. pseudintermedius* (not recommended) *S. schleiferi* (not recommended)	Testing is only indicated for the species listed below: *S. epidermidis* *S. pseudintermedius* *S. schleiferi*	*Staphylococcus* spp. except: *S. aureus* (refer to Table 3G-1) *S. lugdunensis* (refer to Table 3G-1)
Medium	MHA	MHA	CAMHB with 2% NaCl (broth microdilution) MHA with 2% NaCl (agar dilution)
Antimicrobial concentration	30 µg cefoxitin disk	1-µg oxacillin disk	0.5 µg/mL oxacillin
Inoculum	Standard disk diffusion procedure	Standard disk diffusion procedure	Standard broth microdilution procedure or standard agar dilution procedure
Incubation conditions	33 to 35°C; ambient air[c]		
Incubation length	24 hours (may be reported after 18 hours, if resistant)	16–18 hours	24 hours (may be reported after 18 hours, if resistant)
Results	≤ 24 mm = positive for *mecA*-mediated resistance ≥ 25 mm = negative for *mecA*-mediated resistance	≤ 17 mm = positive for *mecA*-mediated resistance ≥ 18 mm = negative for *mecA*-mediated resistance	≥ **1** µg/mL = positive for *mecA*-mediated resistance ≤ **0.5** µg/mL = negative for *mecA*-mediated resistance
Additional testing and reporting	Isolates that test positive for *mecA*-mediated resistance should be reported as methicillin (oxacillin) (not cefoxitin) resistant; other ß-lactam agents, except ceftaroline, should be reported as resistant or should not be reported.[d]		
			For *Staphylococcus* spp., excluding *S. aureus*, *S. lugdunensis*, *S. epidermidis*, *S. pseudintermedius*, and *S. schleiferi*, oxacillin MIC breakpoints may overcall resistance, and some isolates for which the oxacillin MICs are **1**–2 µg/mL may be *mecA* negative. Isolates from serious infections for which oxacillin MICs are **1**–2 µg/mL may be tested for *mecA* or for PBP2a. Isolates that test *mecA* or PBP2a negative should be reported as methicillin (oxacillin) susceptible.
QC recommendations - routine[e]	*S. aureus* ATCC®[f] 25923 – *mecA* negative (zone 23–29 mm)	*S. aureus* ATCC® 25923 – *mecA* negative (zone 18–24 mm)	*S. aureus* ATCC® 29213 – *mecA* negative (MIC 0.12–0.5 µg/mL)
QC recommendations - lot/shipment[g]	N/A	*S. aureus* ATCC® 43300 – *mecA* positive (zone ≤ 24 mm)	*S. aureus* ATCC® 43300 – *mecA* positive (MIC ≥ 8 µg/mL)

Abbreviations: ATCC, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; MRS, methicillin (oxacillin)-resistant *Staphylococcus* spp.; N/A, not applicable.

Table 3G-2. (Continued)

Footnotes

a. **Including members of the *S. aureus* complex (see Table 2C, general comment [2]).**

b. Cefoxitin is tested as a surrogate for detecting *mecA*-mediated methicillin (oxacillin) resistance; **however, recent data suggest that the cefoxitin disk diffusion test may not perform reliably for all species (eg, *S. haemolyticus*).**[1]

c. Testing at temperatures above 35°C may not detect MRS.

d. Testing of other β-lactam agents, except ceftaroline, is not advised.

e. 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

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

g. 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 3G-2

1 **Humphries RM, Magnano P, Burnham CA, et al. Evaluation of surrogate tests for the presence of *mecA*-mediated methicillin resistance in *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus capitis* and *Staphylococcus warneri*. *J Clin Microbiol*. 2020;59(1):e02290-20.**

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

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

Table 3H. 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 enterococci for infection prevention 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.

Table 3H. (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 3H

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

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

Table 3I. Test for Detecting Inducible Clindamycin Resistance in *Staphylococcus spp.*, *Streptococcus pneumoniae*, and *Streptococcus* spp. β-Hemolytic Group[a,b]

Test	ICR			
Test method	**Disk Diffusion (D-zone test)**		**Broth Microdilution**	
Organism group (applies only to organisms resistant to erythromycin and susceptible or intermediate to clindamycin)	All *Staphylococcus* spp.	*S. pneumoniae* and β-hemolytic *Streptococcus* spp.	All *Staphylococcus* spp.[c]	*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) = ICR. Hazy growth within the zone of inhibition around clindamycin = clindamycin resistance, even if no D-zone is apparent.		Any growth = ICR. No growth = no ICR.	

Table 3I. (Continued)

Test	ICR			
Test method	**Disk Diffusion (D-zone test)**		**Broth Microdilution**	
Organism group (applies only to organisms resistant to erythromycin and susceptible or intermediate to clindamycin)	All *Staphylococcus* spp.	*S. pneumoniae* and ß-hemolytic *Streptococcus* spp.	All *Staphylococcus* spp.[c]	*S. pneumoniae* and ß-hemolytic *Streptococcus* spp.
Additional testing and reporting	Report isolates with ICR as "clindamycin resistant." The following comment may be included with the report: "This isolate is presumed to be resistant based on detection of ICR, as determined by testing clindamycin in combination with erythromycin."			
QC recommendations - routine[c]	*S. aureus* ATCC®[d] 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™ or *S. aureus* ATCC® 29213 – no growth	*S. pneumoniae* ATCC® 49619 or *S. aureus* ATCC® BAA-976™ – no growth
QC recommendations - lot/shipment[e]			*S. aureus* ATCC® BAA-977™ – growth	
QC recommendations - supplemental[f]	*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; ICR, inducible clindamycin resistance; LHB, lysed horse blood; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; TSA, tryptic soy agar.

Footnotes

a. Antimicrobial susceptibility testing of ß-hemolytic streptococci does not need to be performed routinely (see general comment [4] in Table 2H-1). When susceptibility testing is clinically indicated, test for ICR in strains that are erythromycin resistant and clindamycin susceptible or intermediate.

b. 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 clindamycin (including ICR) (see comment [12] in Table 2H-1).[1] For isolates that test susceptible to clindamycin (with erythromycin induction), consider adding the following comment to the patient's report: "This group B *Streptococcus* does not demonstrate inducible clindamycin resistance as determined by testing clindamycin in combination with erythromycin."

Table 3I
Test for Inducible Clindamycin Resistance in *Staphylococcus* spp., *Streptococcus pneumoniae*, and *Streptococcus* spp. ß-Hemolytic Group

Table 3I. (Continued)

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[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

d. 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.

e. QC recommendations – lot/shipment

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

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 AST QC programs. See Appendix C, which describes use of QC strains.

References for Table 3I

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. Clinical and Laboratory Standards Institute; 2018.

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

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Table 3J. Test for Detecting 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.

Table 3J. (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 3J

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

2 Simor AE, Phillips E, McGeer A, et al. Randomized controlled trial of chlorhexidine gluconate for washing, intranasal mupirocin, and rifampin and doxycycline versus no treatment for the eradication of methicillin-resistant *Staphylococcus aureus* colonization. *Clin Infect Dis*. 2007;44(2):178-185.

3 Harbarth S, Dharan S, Liassine N, Herrault P, Auckenthaler R, Pittet D. Randomized, placebo-controlled, double-blind trial to evaluate the efficacy of mupirocin for eradicating carriage of methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother*. 1999;43(6):1412-1416.

4 Walker ES, Vasquez JE, Dula R, Bullock H, Sarubbi FA. Mupirocin-resistant, methicillin-resistant *Staphylococcus aureus;* does mupirocin remain effective? *Infect Control Hosp Epidemiol*. 2003;24(5):342-346.

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

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

Table 3K. Test for Detecting 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

Table 3K. (Continued)

Test	Gentamicin HLAR			Streptomycin HLAR		
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 penicillin or ampicillin MICs > 16 µg/mL 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.

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

Table 3K. (Continued)

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.

References for Table 3K

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

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

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

[4] CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. 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	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 25923
Amikacin	30 µg	19-26	20-26	20-26
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	25-31	23-29
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	23-29	24-33
Cefotaxime	30 µg	29-35	18-22	25-31
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	22-29	16-20
Ceftibuten	30 µg	27-35	-	-
Ceftizoxime	30 µg	30-36	12-17	27-35
Ceftobiprole	**5 µg**	**25-31**	**-**	**20-27**
Ceftriaxone	30 µg	29-35	17-23	22-28
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	-	-

Table 4A-1. (Continued)

Antimicrobial Agent	Disk Content	Disk Diffusion QC Ranges, mm		
		Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 25923
Ciprofloxacin	5 µg	29-38	25-33	22-30
Clarithromycin	15 µg	-	-	26-32
Clinafloxacin	5 µg	31-40	27-35	28-37
Clindamycin[c]	2 µg	-	-	24-30
Colistin	10 µg	11-17	11-17	-
Delafloxacin[d]	5 µg	28–35	23–29	32–40
Dirithromycin	15 µg	-	-	18-26
Doripenem	10 µg	27-35	28-35	33-42
Doxycycline	30 µg	18-24	-	23-29
Enoxacin	10 µg	28-36	22-28	22-28
Eravacycline	20 µg	**17-24**	-	19-26
Ertapenem	10 µg	29-36	13-21	24-31
Erythromycin[c]	15 µg	-	-	22-30
Faropenem	5 µg	20-26	-	27-34
Fleroxacin	5 µg	28-34	12-20	21-27
Fosfomycin[e]	200 µg	22-30	-	25-33
Fusidic acid	10 µg	-	-	24-32
Garenoxacin	5 µg	28-35	19-25	30-36
Gatifloxacin	5 µg	30-37	20-28	27-33
Gemifloxacin	5 µg	29-36	19-25	27-33
Gentamicin[f]	10 µg	19-26	17-23	19-27
Gepotidacin	10 µg	18-26	-	23-29
Grepafloxacin	5 µg	28-36	20-27	26-31
Iclaprim	5 µg	14-22	-	25-33
Imipenem[g]	10 µg	26-32	20-28	-
Kanamycin	30 µg	17-25	-	19-26
Lefamulin	20 µg	-	-	26-32
Levofloxacin	5 µg	29-37	19-26	25-30
Levonadifloxacin	10 µg	27-33[d]	17-23[d]	32-39[d]
Linezolid	30 µg	-	-	25-32[h]
Lomefloxacin	10 µg	27-33	22-28	23-29
Loracarbef	30 µg	23-29	-	23-31
Mecillinam	10 µg	24-30	-	-
Meropenem	10 µg	28-35	27-33	29-37
Minocycline	30 µg	19-25	-	25-30
Moxalactam	30 µg	28-35	17-25	18-24

Table 4A-1. (Continued)

Antimicrobial Agent	Disk Content	Disk Diffusion QC Ranges, mm		
		Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 25923
Moxifloxacin	5 μg	28-35	17-25	28-35
Nafcillin	1 μg	-	-	16-22
Nafithromycin	15 μg	-	-	25-31[d]
Nalidixic acid	30 μg	22-28	-	-
Netilmicin	30 μg	22-30	17-23	22-31
Nitrofurantoin	300 μg	20-25	-	18-22
Norfloxacin	10 μg	28-35	22-29	17-28
Ofloxacin	5 μg	29-33	17-21	24-28
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	15-21	19-25
Polymyxin B	300 units	13-19	14-18	-
Quinupristin-dalfopristin	15 μg	-	-	21-28
Razupenem	10 μg	21-26	-	-[i]
Rifampin	5 μg	8-10	-	26-34
Solithromycin	15 μg	-	-	22-30
Sparfloxacin	5 μg	30-38	21-29	27-33
Streptomycin[f]	10 μg	12-20	-	14-22
Sulfisoxazole[j]	250 μg or 300 μg	15-23	-	24-34
Sulopenem	2 μg	24-30[d]	-	-
Tebipenem[g]	10 μg	30-37	20-26	-
Tedizolid[k]	2 μg	-	-	18-24[h]
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	9-13	20-25
Tobramycin	10 μg	18-26	20-26	19-29
Trimethoprim[j]	5 μg	21-28	-	19-26
Trimethoprim-sulfamethoxazole[j]	1.25/23.75 μg	23-29	-	24-32
Trospectomycin	30 μg	10-16	-	15-20
Trovafloxacin	10 μg	29-36	21-27	29-35
Ulifloxacin (prulifloxacin)[l]	5 μg	32-38	27-33	20-26
Vancomycin	30 μg	-	-	17-21

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

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 (ICR) (ie, a positive D-zone test), whereas *S. aureus* ATCC® BAA-976™ should not demonstrate ICR. *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. QC ranges were established using data from only one disk manufacturer. Disks from other manufacturers were not available at the time of testing.

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

f. 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).

g. *Klebsiella pneumoniae* ATCC® 700603 is a supplemental QC strain for testing QC of imipenem (25–33 mm) and tebipenem (26–32 mm).

h. Zones of inhibition for linezolid and tedizolid with *S. aureus* ATCC® 25923 should be read using transmitted light.

i. 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.

j. 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.

k. *E. faecalis* ATCC® 29212 is a supplemental QC strain for testing QC of tedizolid (14–21 mm) to assist with reading.

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

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

Reference for Table 4A-1

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

Table 4A-2. Disk Diffusion QC Ranges for Nonfastidious Organisms and ß-Lactam Combination Agents[a]

Antimicrobial Agent	Disk Content	QC Organisms and Characteristics								
		Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 25923	*Escherichia coli* ATCC® 35218[c,d]	*Klebsiella pneumoniae* ATCC® 700603[c,d]	*Escherichia coli* NCTC 13353[c,d]	*Klebsiella pneumoniae* ATCC® BAA-1705™[c,d]	*Klebsiella pneumoniae* ATCC® BAA-2814™	*Acinetobacter baumannii* NCTC 13304[c,d]
		ß-lactamase negative	Inducible AmpC	ß-lactamase negative, *mecA* 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	OXA-27
		Zone Diameter 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	31-37	23-29	6-15[f]	-	-	6-16[f]
Cefepime-enmetazobactam[e]	30/20 µg	32-38	26-32	-	32-38	26-32	27-33	-	-	-
Cefepime-taniborbactam	30/20 µg	31-37	25-31	-	31-37	24-31	24-30	22-27	-	-
Cefepime-tazobactam	30/20 µg	32-37	27-31	24-30	-	25-30[e]	27-31	-	-	-
Cefepime-zidebactam	30/30 µg	33-40	29-35	-	-	28-34	29-35	-	-	19-25
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	30/20 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	-	-	-	-
Imipenem	10 µg	26-32	20-28	-	-	25-33	-	11-22	6-14	-
Imipenem-relebactam[e,g]	10/25 µg	27-33	26-31	-	-	26-32	-	23-29	22-28	-
Meropenem[f]	10 µg	28-35	27-33	29-37	-	-	-	11-18[e]	6[e]	-

Table 4A-2. (Continued)

Antimicrobial Agent	Disk Content	QC Organisms and Characteristics								
		Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 25923	*Escherichia coli* ATCC® 35218[c,d]	*Klebsiella pneumoniae* ATCC® 700603[c,d]	*Escherichia coli* NCTC 13353[c,d]	*Klebsiella pneumoniae* ATCC® BAA-1705™[c,d]	*Klebsiella pneumoniae* ATCC® BAA-2814™	*Acinetobacter baumannii* NCTC 13304[c,d]
		β-lactamase negative	Inducible AmpC	β-lactamase negative, *mecA* 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	OXA-27
		Zone Diameter QC Ranges, mm								
Meropenem-vaborbactam[g]	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	-	-	-	-	-
Sulbactam-durlobactam	10/10 µg	26-32	-	-	-	-	-	-	-	24-30
Ticarcillin	75 µg	24-30	21-27	-	6	-	-	-	-	-
Ticarcillin-clavulanate	75/10 µg	24-30	20-28	29-37	21-25	-	-	-	-	-

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

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.[c,d]

Footnotes

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

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. 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.

d. 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.

Table 4A-2. (Continued)

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

f. If discrete colonies or a haze of growth are present inside the zone of inhibition, measure the colony-free inner zone.

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

References for Table 4A-2

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

2 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. 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	-	30-38	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

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]
Erythromycin	15 µg	–	–	–	25-30
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	–	–	32-40	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	2 µg	–	–	–	18-25
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

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 MH-F agar for *S. pneumoniae* only
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; MH-F agar, Mueller-Hinton fastidious agar; QC, quality control.

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 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.

Test Modification	Recommended QC Frequency			Comments
	1 Day	5 Days	15-Replicate Plan or 20- or 30-Day Plan	
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.

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™. Clinical and Laboratory Standards Institute; 2011.

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

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 (refer to M02,[1] Subchapter 4.4). If using lyophilized strains, follow the maintenance recommendations of the manufacturer.

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
β-LACTAMS				
β-lactam combination agents	*A. baumannii* ATCC®[a] 13304 *E. coli* ATCC® 35218 *E. coli* ATCC® 13353 *K. pneumoniae* ATCC® 700603 *K. pneumoniae* ATCC® BAA-1705™	Zone too large or susceptible for single β-lactam agent; in range for combination β-lactam agent	Spontaneous loss of the plasmid encoding the ß-lactamase	Obtain new frozen or lyophilized stock culture. Use other routine QC strains (if available). These strains should be stored at −60°C or below, and frequent subcultures should be avoided. **NOTE:** *K. pneumoniae* BAA-2814™ is stable and does not require QC integrity check.
β-lactam combination agents	*A. baumannii* ATCC® 13304 *E. coli* ATCC® 35218 *E. coli* ATCC® 13353 *K. pneumoniae* ATCC® 700603 *K. pneumoniae* ATCC® BAA-1705™ *K. pneumoniae* ATCC® BAA-2814™	Zone too small or resistant for both the single β-lactam agent and the combination β-lactam agent	Antimicrobial agent is degrading.	Use alternative lot of test materials. Check storage and package integrity. Imipenem and clavulanate are especially labile.
Carbenicillin	*P. aeruginosa* ATCC® 27853	Zone too small	QC strain develops resistance after repeated subculture.	See general comment (1) on QC strain maintenance.
Cefepime	*A. baumannii* NCTC 13304 *E. coli* NCTC 13353	QC strain integrity test	Discrete colonies may grow within the zone of inhibition when this organism is tested with cefepime 30-µg disk.	If this occurs, measure the colony-free inner zone.
Imipenem	*K. pneumoniae* ATCC® BAA-1705™ *K. pneumoniae* ATCC® BAA-2814™	QC strain integrity test	Discrete colonies may grow within the zone of inhibition when this organism is tested with cefepime. 30-µg disk.	If this occurs, measure the colony-free inner zone.
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

Table 4D. (Continued)

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
NON-β-LACTAMS				
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.
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
Tedizolid	*E. faecalis* ATCC® 29212	Zone with *Enterococcus* spp. is difficult to read	Light growth on MHA	*E. faecalis* ATCC® 29212 is provided as supplemental QC to assist in personnel training and assessment of proper reading. Measure zone edge where there is a significant decrease in density of growth when using transmitted light as illustrated in the photographs.[b]
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.

Table 4D. (Continued)

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
ALL AGENTS				
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	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	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.
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	Any	QC results from one strain are out of range, but results from other QC strain(s) is in range with the same antimicrobial agent.	One QC strain 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 agent(s).
Various	Any	QC results from two 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; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; NCTC, National Collection of Type Cultures; pH, negative logarithm of hydrogen ion concentration; QC, quality control.

Table 4D. (Continued)

Footnotes

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

b. Figure 1 shows examples of tedizolid disk diffusion results for *E. faecalis*.

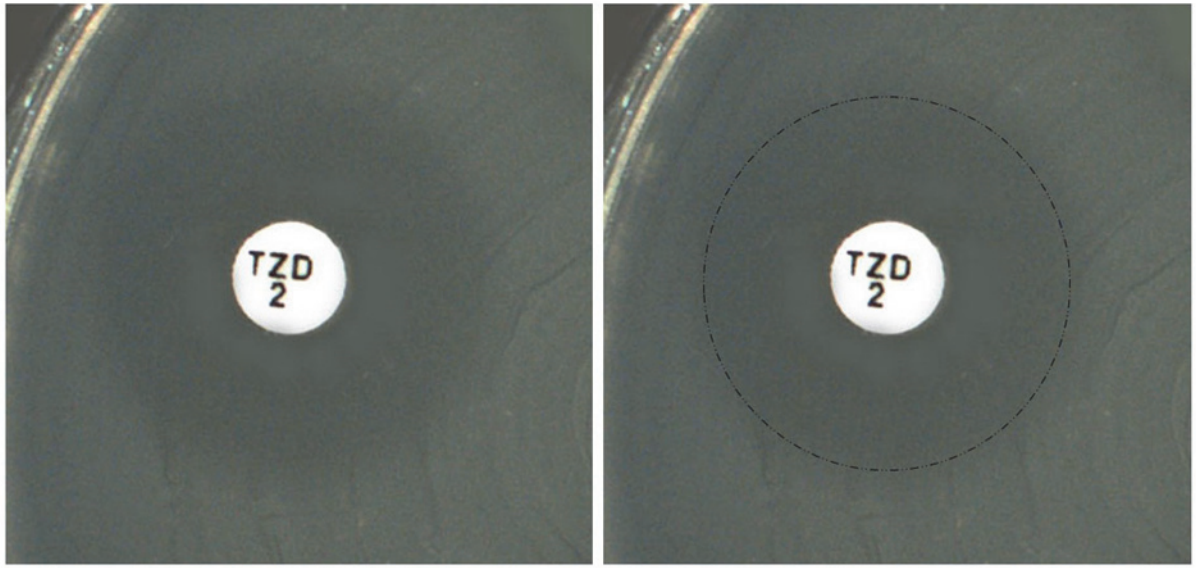

Figure 1. Measuring the Tedizolid Zone for *E. faecalis* ATCC® 29212 When Light Growth Is Observed

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

Reference for Table 4D

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

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

Table 5A-1. (Continued)

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

Table 5A-1. (Continued)

Antimicrobial Agent	MIC QC Ranges, µg/mL			
	Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 29213	*Enterococcus faecalis* ATCC® 29212
Mecillinam	0.03-0.25[n]	-	-	-
Meropenem	0.008-0.06	0.12-1	0.03-0.12	2-8
Minocycline[f]	0.25-1	-	0.06-0.5	1-4
Moxalactam	0.12-0.5	8-32	4-16	-
Moxifloxacin	0.008-0.06	1-8	0.016-0.12	0.06-0.5
Nafcillin	-	-	0.12-0.5	2-8
Nafithromycin	-	-	0.06-0.25	0.016-0.12
Nalidixic acid[f]	1-4	-	-	-
Netilmicin	≤0.5-1	0.5-8	≤0.25	4-16
Nitrofurantoin	4-16	-	8-32	4-16
Norfloxacin	0.03-0.12	1-4	0.5-2	2-8
Ofloxacin	0.016-0.12	1-8	0.12-1	1-4
Omadacycline[o]	0.25-2	-	0.12-1	0.06-0.5
Oritavancin[h]	-	-	0.016-0.12	0.008-0.03
Oxacillin	-	-	0.12-0.5	8-32
Ozenoxacin	-	-	0.001-0.004	0.015-0.06
Penicillin	-	-	0.25-2	1-4
Pexiganan	2-8	2-16	8-32	16-64
Piperacillin	1-4	1-8	1-4	1-4
Plazomicin	0.25-2	1-4	0.25-2	-
Polymyxin B	0.25-2	0.5-2	-	-
Quinupristin-dalfopristin	-	-	0.25-1	2-8
Razupenem	0.06-0.5	-	0.008-0.03	0.25-1
Rifampin	4-16	16-64	0.004-0.016	0.5-4
Solithromycin	-	-	0.03-0.12	0.016-0.06
Sparfloxacin	0.004-0.016	0.5-2	0.03-0.12	0.12-0.5
Sulfisoxazole[f,p]	8-32	-	32-128	32-128
Sulopenem	0.016-0.06	-	0.016-0.12	2-8
Tebipenem	0.008-0.03	1-8	0.015-0.06	0.25-1
Tedizolid[q]	-	-	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	0.5-2	8-32	0.12-1	8-32
Ticarcillin	4-16	8-32	2-8	16-64
Tigecycline[o]	0.03-0.25	-	0.03-0.25	0.03-0.12
Tobramycin	0.25-1	0.25-1	0.12-1	8-32

Table 5A-1. (Continued)

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

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

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 CAMHB is iron depleted. Chelation is used for iron depletion, which also removes other cations (ie, calcium, magnesium, and zinc). Following this process, cations are added back to concentrations of calcium 20–25 mg/L, magnesium 10–12.5 mg/L, and zinc 0.5–1.0 mg/L.

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 (ICR) 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 ICR (ie, growth in the well), whereas *S. aureus* ATCC® 29213 and *S. aureus* ATCC® BAA-976™ should not demonstrate ICR (ie, no growth in the well).

Table 5A-1. (Continued)

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

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. Exebacase QC ranges reflect MICs obtained when CAMHB is supplemented with 25% horse serum plus 0.5 mM DL-dithiothreitol (pH 7.2–7.4).

k. 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.

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

m. 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.

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

o. 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.

p. Very medium-dependent, especially with enterococci.

q. 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.

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

s. For QC organisms for vancomycin screen test for enterococci, see Table **3H**.

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.

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

Antimicrobial Agent	QC Organisms and Characteristics									
	Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 29213	*Enterococcus faecalis* ATCC® 29212	*Escherichia coli* ATCC® 35218[c,d]	*Klebsiella pneumoniae* ATCC® 700603[c,d]	*Escherichia coli* NCTC 13353[c,d]	*Klebsiella pneumoniae* ATCC® BAA-1705™[c,d]	*Klebsiella pneumoniae* ATCC® BAA-2814™	*Acinetobacter baumannii* NCTC 13304[c,d]
	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)[e]	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)[e]	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	-	-	> 128	-
Aztreonam-avibactam	0.03/4-0.12/4	2/4-8/4	-	-	0.016/4-0.06/4	0.06/4-0.5/4	-	-	-	-
Aztreonam-nacubactam (1:1)[e]	**0.06/0.06-0.25/0.25**	**2/2-8/8**	**-**	**-**	**-**	**0.5/0.5-2/2**	**-**	**-**	**0.5/0.5-2/2**	**-**
Cefepime	0.016-0.12	0.5-4	1-4	-	0.008-0.06	0.5-2	≥ 64	-	> 32	16-128
Cefepime-enmetazobactam	0.03/8-0.12/8	0.5/8-2/8	-	-	0.008/8-0.06/8	0.12/8-0.5/8	0.03/8-0.12/8	-	-	-
Cefepime-nacubactam (1:1)	**0.016/0.016-0.12/0.12**	**0.5/0.5-2/2**	**-**	**-**	**-**	**0.12/0.12-0.5/0.5**	**-**	**-**	**0.5/0.5-2/2**	**-**
Cefepime-taniborbactam	0.03/4-0.12/4	0.5/4-4/4	-	-	0.016/4-0.06/4	0.12/4-0.5/4	0.12/4-1/4	0.12/4-0.5/4	-	-
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[f]	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	–	0.12-0.5	4-32	32-128	-	-	-
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	-	-	-	-

Table 5A-2. (Continued)

Antimicrobial Agent	QC Organisms and Characteristics									
	Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 29213	*Enterococcus faecalis* ATCC® 29212	*Escherichia coli* ATCC® 35218[c,d]	*Klebsiella pneumoniae* ATCC 700603[c,d]	*Escherichia coli* NCTC 13353[c,d]	*Klebsiella pneumoniae* ATCC® BAA-1705™[c,d]	*Klebsiella pneumoniae* ATCC® BAA-2814™	*A. baumannii* NCTC 13304[c,d]
	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									
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	–	-	-	-	-	-	-
Durlobactam	0.12-0.5	–	–	–	–	–	–	–	–	32-128
Imipenem	0.06-0.25	1-4	0.016-0.06	0.5-2	-	0.03-0.25	-	4-16	16-64	-
Imipenem-relebactam[e]	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-**0.5**/4	-
Meropenem	0.008-0.06	0.12-1	0.03-0.12	2-8	0.008-0.06	-	-	8-64	32-256	-
Meropenem-nacubactam (1:1)	0.015/0.015-0.06/0.06	0.12/0.12-1/1	-	-	-	-	-	-	0.5/0.5-2/2	-
Meropenem-vaborbactam[e]	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	-
Nacubactam[f]	0.5-4	64-256	-	-	-	-	-	-	0.5-4	-
Piperacillin	1-4	1-8	1-4	1-4	> 64	-	-	-	-	-
Piperacillin-tazobactam[e]	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	-	-	-	-
Sulbactam	16-64					32-128				16-64
Sulbactam-durlobactam	-	-	-	-	-	-	-	-	-	0.5-2
Ticarcillin	4-16	8-32	2-8	16-64	> 128	> 256	-	-	-	-
Ticarcillin-clavulanate[e]	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	-	-	-	-

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. (green)

Test one of these agents by a disk diffusion or MIC method to confirm the integrity of the respective QC strain.[c,d] (orange)

Table 5A-2. (Continued)

Footnotes

a. Unsupplemented Mueller-Hinton medium (cation-adjusted if broth). See Table 5A-1 for QC ranges for combination agents from other drug classes.

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. 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 ß-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.

d. To confirm the integrity of the QC strain, test one of the single ß-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 ß-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 ß-lactam combination agents (eg, ampicillin panel concentrations 1–16 µg/mL; ampicillin Enterobacterales breakpoints [µg/mL]: ≤8 [S], 16 [I], ≥32 [R]; MIC of >16 µg/ml [R] would be acceptable for *K. pneumoniae* ATCC® 700603).

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

f. Not tested as a single agent routinely.

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

References for Table 5A-2

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

2 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. 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[g]	-	-	0.008-0.03

Table 5B. (Continued)

Antimicrobial Agent	MIC QC Ranges, µg/mL		
	Haemophilus influenzae ATCC®[a] 49247	*Haemophilus influenzae* ATCC® 49766	*Streptococcus pneumoniae* ATCC® 49619
Daptomycin[h]	-	-	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[i]	0.5-2	-	0.016-0.12

Table 5B. (Continued)

Antimicrobial Agent	MIC QC Ranges, µg/mL		
	Haemophilus influenzae ATCC®[a] 49247	*Haemophilus influenzae* ATCC® 49766	*Streptococcus pneumoniae* ATCC® 49619
Oritavancin[g]	-	-	0.001-0.004
Ozenoxacin	-	-	0.008-0.06
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[g]	-	-	0.004-0.016
Telithromycin	1-4	-	0.004-0.03
Tetracycline	4-32	-	0.06-0.5
Tigecycline[i]	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
Zoliflodacin	0.12-1	-	0.12-0.5

MIC 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.

Table 5B. (Continued)

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

h. 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.

i. 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.

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

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
Zoliflodacin	0.06-0.5

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.

Footnote

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

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	-
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

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.

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.

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.

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.

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

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 documents EP23[1] and M52[2]). 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.

Test Modification	Recommended QC Frequency			Comments
	1 Day	5 Days	15-Replicate Plan or 20- or 30-Day Plan	
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.

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,[3] 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™. Clinical and Laboratory Standards Institute; 2011.

2 CLSI. *Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems*. 1st ed. CLSI guideline M52. Clinical and Laboratory Standards Institute; 2015.

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

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 (refer to M07,[1] Subchapter 4.4). If using lyophilized strains, follow the maintenance recommendations of the manufacturer.

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
β-LACTAMS				
β-lactam combination agents	*A. baumannii* ATCC®[a] 13304 *E. coli* ATCC® 35218 *E. coli* ATCC® 13353 *K. pneumoniae* ATCC® 700603 *K. pneumoniae* ATCC® BAA-1705™	MIC too low or susceptible for single β-lactam agent; in range for combination β-lactam agent	Spontaneous loss of the plasmid encoding the β-lactamase	Obtain new frozen or lyophilized stock culture. Use other routine QC strain (if available). These strains should be stored at −60°C or below, and frequent subcultures should be avoided. NOTE: *K. pneumoniae* ATCC® BAA-2814™ is stable and does not require QC integrity check.
β-lactam combination agents	*A. baumannii* ATCC® 13304 *E. coli* ATCC® 35218 *E. coli* ATCC® 13353 *K. pneumoniae* ATCC® 700603 *K. pneumoniae* ATCC® BAA-1705™ *K. pneumoniae* ATCC® BAA-2814™	MIC too high or resistant for both the single β-lactam agent and the combination β-lactam agent	Antimicrobial agent is degrading.	Use alternative lot of test materials. Check storage and package integrity. Imipenem and clavulanate are especially labile.
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.

Table 5G. (Continued)

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
Β-LACTAMS (Continued)				
Penicillins	Any	MIC too low	pH of media too low	Acceptable pH range = 7.2–7.4 Avoid CO_2 incubation, which lowers pH.
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 **high**	Ca++ and/or Mg++ content too **high**	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

Table 5G. (Continued)

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
NON-β-LACTAMS (Continued)				
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	***S. pneumoniae* ATCC® 49619**	**MICs too low** **Light growth**	**Inoculum source plate too old and contains too many nonviable cells. Plate used to prepare inoculum should be incubated 18–20 hours.**	**Subculture QC strain and repeat QC test, or retrieve new QC strain from stock.**
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 5×10^5 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 5×10^5 CFU/mL; see M07,[1] Subchapter 3.8).

Table 5G. (Continued)

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
ALL AGENTS (Continued)				
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	QC results from one strain are 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	QC results from two 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	QC results from one strain are 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.

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; pH, negative logarithm of hydrogen ion concentration; QC, quality control.

Footnote

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

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

References for Table 5G

1. CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. 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.

Table 6A. Solvents and Diluents for Preparing Stock Solutions of Antimicrobial Agents[a]

Antimicrobial Agent	Solvent[b] Unless otherwise stated, use a minimum amount of the listed solvent to solubilize the antimicrobial powder.	Diluent[b] 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[a,c]	Broth media
Azlocillin	Water	Water
Aztreonam	Saturated solution sodium bicarbonate	Water
Besifloxacin	Methanol	Water
Biapenem	Saline[d]	Saline[d]
Cadazolid	DMSO[a]	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 or water
Cefetamet	Phosphate buffer, pH 6, 0.1 mol/L	Water
Cefiderocol	Saline[d]	Saline[d]
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[a]	Water
Cefoxitin	Water	Water
Cefpodoxime	0.10% (11.9 mmol/L) aqueous sodium bicarbonate	Water
Cefprozil	Water	Water
Ceftaroline	DMSO[a] to 30% of total volume	Saline[d]
Ceftazidime	Sodium carbonate[e]	Water
Ceftibuten	1/10 volume of DMSO[a]	Water
Ceftizoxime	Water	Water
Ceftobiprole	DMSO plus glacial acetic acid[a,f]	Water, vortex vigorously

Table 6A. (Continued)

Antimicrobial Agent	Solvent[b] Unless otherwise stated, use a minimum amount of the listed solvent to solubilize the antimicrobial powder.	Diluent[b] Finish diluting the final stock solution as stated below.
Ceftolozane	Water or saline[d]	Water or saline[d]
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[a] or glacial acetic acid[a,c]	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[g]	Water	Water
Dalbavancin	DMSO[a]	DMSO[a,h]
Daptomycin	Water	Water
Delafloxacin	1/2 volume of water, then 0.1 mol/L NaOH dropwise to dissolve	Water
Dirithromycin	Glacial acetic acid[c]	Water
Doripenem	Saline[d]	Saline[d]
Doxycycline	Water	Water
Durlobactam	Water	Water
Enoxacin	1/2 volume of water, then 0.1 mol/L NaOH dropwise to dissolve	Water
Enmetazobactam	Water	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[a,c]	Water
Exebacase	Supplied as a frozen stock in a buffer containing 20 mM L-histidine and 5% D-sorbitol, pH 7	CAMHB supplemented with 25% horse serum plus 0.5 mM DL-dithiothreitol (pH 7.2–7.4)
Faropenem	Water	Water
Fidaxomicin	DMSO[a]	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

Table 6A. (Continued)

Antimicrobial Agent	Solvent[b]	Diluent[b]
	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	DMSOa	Water
Iclaprim	DMSOa	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
Metronidazole	DMSO[a]	Water
Minocycline	Water	Water
Moxalactam (diammonium salt)[i]	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
Nacubactam	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[a,j]	DMSO[a,j]
Nitrofurantoin[k]	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
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[l]	0.002% polysorbate-80 in water[l]

Table 6A. (Continued)

Antimicrobial Agent	Solvent[b] Unless otherwise stated, use a minimum amount of the listed solvent to solubilize the antimicrobial powder.	Diluent[b] Finish diluting the final stock solution as stated below.
Oxacillin	Water	Water
Ozenoxacin	10% volume of water, then 1M NaOH (8% of final volume)	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[a]	DMSO[a]
Rifampin	Methanol[a] (maximum concentration = 640 µg/mL)	Water (with stirring)
Rifaximin	Methanol[a]	0.1 M phosphate buffer, pH 7.4 + 0.45% sodium dodecyl sulfate
Secnidazole	DMSO[a]	Water
Solithromycin	Glacial acetic acid[c]	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[m]	0.01 M phosphate buffer, pH 7.2, vortex to dissolve	0.01 M phosphate buffer, pH 7.2
Surotomycin	Water	Water
Taniborbactam	Water	Water
Tazobactam	Water	Water
Tebipenem	Water	Water
Tedizolid	DMSO[a]	DMSO[a,n]
Teicoplanin	Water	Water
Telavancin	DMSO[a]	DMSO[a,h]
Telithromycin	Glacial acetic acid[a,c]	Water

Table 6A. (Continued)

Antimicrobial Agent	Solvent[b] Unless otherwise stated, use a minimum amount of the listed solvent to solubilize the antimicrobial powder.	Diluent[b] Finish diluting the final stock solution as stated below.
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
Tigecycline	Water	Water
Tinidazole	DMSO[a,j]	Water
Tizoxanide	DMSO[a,j]	DMSO[a,j]
Tobramycin	Water	Water
Trimethoprim	0.05 mol/L lactic[a] or hydrochloric[a] acid, 10% of final volume	Water (may need heat)
Trimethoprim (if lactate)	Water	Water
Trospectomycin	Water	Water
Ulifloxacin (prulifloxacin)	DMSO[a]	Water
Vaborbactam	90% DMSO[a]/10% water	Water
Vancomycin	Water	Water
Zidebactam	Water	Water
Zoliflodacin	DMSO	Water

Abbreviations: CAMHB, cation-adjusted Mueller-Hinton broth; DMSO, dimethyl sulfoxide; pH, negative logarithm of hydrogen ion concentration.

Footnotes

a. 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.

b. Although these solvents and diluents are recommended, users should always confirm with the manufacturer.

c. 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.

d. Saline – a solution of 0.85% to 0.9% NaCl (w/v).

e. 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.

f. 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.

g. The formulation of colistin reference standard powder used in antimicrobial susceptibility tests is colistin sulfate and not colistin methane sulfonate (sulfomethate).

Table 6A. (Continued)

h. 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 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.

i. 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

j. 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

k. Alternatively, nitrofurantoin is dissolved in DMSO.

l. 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%.

m. Must be made fresh on the day of use.

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%. Also see Table 8B.

Table 6B. Preparing 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

[1] Geddes AM, Gould IM. Benzylpenicillin (penicillin G). In: Grayson ML, ed. *Kucers' The Use of Antibiotics: A Clinical Review of Antibacterial, Antifungal, Antiparasitic and Antiviral Drugs*. 6th ed. Boca Raton, FL: CRC Press, Taylor & Francis Group; 2010:5-58.

[2] Polymyxins. In: Kucers A, Crowe SM, Grayson ML, Hoy JF, eds. *The Use of Antibiotics: A Clinical Review of Antibacterial, Antifungal, Antiparasitic and Antiviral Drugs*. 5th ed. Oxford, UK: Butterworth-Heinemann; 1997:667-675.

[3] United States Department of Agriculture, Food Safety and Inspection Service, Office of Public Health Science, Laboratory QA/QC Division. *Bioassay for the detection, identification and quantitation of antimicrobial residues in meat and poultry tissue*. Microbiology Laboratory Guidebook (MLG) 34.03; 2011.

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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 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 2-fold 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 2-fold 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.
Aztreonam-nacubactam	**1:1 ratio (aztreonam:nacubactam)**	**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 aztreonam and 2560 μg/mL for nacubactam. Combine equal amounts of each to the first dilution tube, which will then contain 1280/1280 μg/mL of the combination. Prepare 2-fold serial dilutions and dilute each 1:10 with broth to achieve the final concentration in the microdilution wells.**
Cefepime-enmetazobactam	Fixed concentration of enmetazobactam at 8 mg/L	Prepare 10× starting concentration of cefepime at twice the concentration needed and dilute as usual using serial 2-fold dilutions. Add an equal volume of enmetazobactam 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 2-fold increments down to the final concentration needed in the panel. Prepare a stock concentration of enmetazobactam at 160 μg/mL. Then add an equal volume of the enmetazobactam 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 enmetazobactam = 10 mL of 1280/80 μg/mL cefepime-enmetazobactam. Dilute 1:10 with broth to achieve the final concentration in the microdilution wells.

Table 6C. (Continued)

Antimicrobial Agent	Combination Tested	Preparation	Example
Cefepime-nacubactam	**1:1 ratio (cefepime:nacubactam)**	**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 nacubactam. Combine equal amounts of each to the first dilution tube, which will then contain 1280/1280 µg/mL of the combination. Prepare 2-fold serial dilutions and dilute each 1:10 with broth to achieve the final concentration in the microdilution wells.**
Cefepime-taniborbactam	Fixed concentration of taniborbactam at 4 µg/mL	Prepare 10x starting concentration of cefepime at twice the concentration needed and dilute as usual using serial 2-fold dilutions. Add an equal volume of taniborbactam 80 µg/mL to each of the diluted tubes.	For a starting concentration of 128/4 in the panel, prepare a 10x stock concentration of cefepime at 2560 µg/mL and dilute by serial 2-fold increments down to the final concentration needed in the panel. Prepare a stock concentration of taniborbactam at 80 µg/mL. Then add an equal volume of the taniborbactam 80 µg/mL solution to each diluted tube of cefepime. For example, 5 mL of 2560 µg/mL cefepime + 5 mL of 80 µg/mL taniborbactam = 10 mL of 1280/40 µg/mL cefepime-taniborbactam. 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 2-fold 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 2-fold 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 2-fold 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.	
Ceftolozane-tazobactam	Fixed concentration of tazobactam at 4 µg/mL	Same as aztreonam-avibactam.	

Table 6C. (Continued)

Antimicrobial Agent	Combination Tested	Preparation	Example
Imipenem-relebactam	Fixed concentration of relebactam at 4 µg/mL	Same as aztreonam-avibactam.	
Meropenem-nacubactam	1:1 ratio (meropenem:nacubactam)	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 meropenem and 2560 µg/mL for nacubactam. Combine equal amounts of each to the first dilution tube, which will then contain 1280/1280 µg/mL of the combination. Prepare 2-fold serial dilutions and dilute each 1:10 with broth to achieve the final concentration in the microdilution wells.
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 2-fold 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 2-fold 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.	
Sulbactam-durlobactam	Fixed concentration of durlobactam at 4 ug/mL	Prepare 10× starting concentration of sulbactam at twice the concentration needed and dilute as usual using serial 2-fold dilutions. Add an equal volume of durlobactam 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 sulbactam at 2560 µg/mL and dilute by serial 2-fold increments down to the final concentration needed. Prepare a stock concentration of durlobactam at 80 µg/mL. Then add an equal volume of the durlobactam 80 µg/mL solution to each diluted tube of sulbactam. For example, 5 mL of 2560 µg/mL sulbactam + 5 mL of 80 µg/mL clavulanate = 10 mL of 1280/40 µg/mL sulbactam-durlobactam. Dilute 1:10 with broth to achieve the final concentration in microdilution wells.
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 2-fold 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 2-fold 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.

Table 6C. (Continued)

Antimicrobial Agent	Combination Tested	Preparation	Example
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 2-fold 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.		

NOTE 1: To prepare intermediate dilutions of antimicrobial agents, a convenient formula to use is $C_1 \bullet V_1 = C_2 \bullet V_2$, 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. For example, to prepare 20 mL of a 40 µg/mL solution from a 1280 µg/mL stock solution:

$$C_1 \bullet V_1 = C_2 \bullet V_2$$

$$1280\ \mu g/mL \bullet V_1 = 40\ \mu g/mL \bullet 20\ mL$$

$$V_1 = \frac{40\ \mu g/mL \bullet 20\ mL}{1280\ \mu g/mL}$$

$$V_1 = 0.625\ mL$$

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.

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

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.

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.

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:+.

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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 Antimicrobial Susceptibility Test Results and Organism Identification for Agents Approved by the US Food and Drug Administration for Clinical Use

Organism or Organism Group	Antimicrobial Class/Subclass	Antimicrobial Agent(s) and 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 generally considered of epidemiological concern**
			Action Steps:		
			• Confirm ID and susceptibility.[a] • Report to infection prevention. • Check with public health department to determine appropriate reporting and isolate referral procedures. • Save isolate. **NOTE:** It may be appropriate to notify infection prevention of preliminary findings before confirmation of results.	• Confirm ID and susceptibility if uncommon in the institution.[a] • Check with infection prevention in the facility to determine if special reporting procedures or additional actions are needed. • Check with public health department to determine appropriate reporting and isolate referral procedures.	• Confirm ID and susceptibility if uncommon in the institution.[a] • Check with infection prevention in the facility to determine if special reporting procedures or additional action are needed.
Any Enterobacterales	β-lactam combination agents	Ceftazidime-avibactam - R		X	
		Meropenem-vaborbactam - I or R		X	
	Carbapenems	Any carbapenem - I or R[b]		X	
	Aminoglycosides	Amikacin, gentamicin, and tobramycin - R			X
		Plazomicin - R (except *P. mirabilis*)	X		
	Lipopeptides	Colistin/polymyxin B - R[c]	X		

Appendix A. (Continued)

Organism or Organism Group	Antimicrobial Class/Subclass	Antimicrobial Agent(s) and 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 generally considered of epidemiological concern
Escherichia coli, Klebsiella pneumoniae, K. oxytoca, and *Proteus mirabilis*	Cephems	Cephalosporin III/IV – I/SDD or R			X
Salmonella and *Shigella* spp.[d]	Cephems	Cephalosporin III – I or R		X	
	Macrolides	Azithromycin – NWT or R		X	
	Fluoroquinolones	Any fluoroquinolone – I or R		X	
Acinetobacter baumannii complex	Carbapenems	Any carbapenem[c] – I or R			X
	Lipopeptides	Colistin/polymyxin B – R	X		
Pseudomonas aeruginosa	β-lactam combination agents	Ceftolozane-tazobactam – I or R		X	
	Carbapenems	Any carbapenem[c]– I or R			X
	Aminoglycosides	Amikacin and gentamicin and tobramycin – R			X
	Lipopeptides	Colistin/polymyxin B – R	X		
Stenotrophomonas maltophilia	Folate pathway antagonists	Trimethoprim-sulfamethoxazole – I or R			X

Appendix A. (Continued)

Organism or Organism Group	Antimicrobial Class/Subclass	Antimicrobial Agent(s) and 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 generally considered of epidemiological concern
Haemophilus influenzae	Penicillins	Ampicillin - R and β-lactamase negative		X	
	β-lactam combination agents	Amoxicillin-clavulanate - R **Ceftolozane-tazobactam - NS**	 **X**	X	
	Cephems	Cephalosporin III/IV - NS Ceftaroline - NS	X		
	Carbapenems	Any carbapenem - NS	X		
	Fluoroquinolones	Any fluoroquinolone - NS	X		
	Pleuromutilins	**Lefamulin - NS**	**X**		
Neisseria gonorrhoeae	Cephems	Cephalosporin III/IV - NS		X	
	Macrolides	Azithromycin - NS			X
	Fluoroquinolones	Ciprofloxacin - I or R			X
Enterococcus spp.	Glycopeptides	Vancomycin - R[c]			X
	Lipoglycopeptides (Vancomycin-susceptible *E. faecalis* only)	Dalbavancin - NS Oritavancin - NS Telavancin - NS	X		
	Lipopeptides	Daptomycin - SDD, I, or R		X	
	Oxazolidinones	Linezolid - R Tedizolid - NS		X	
	Aminoglycosides	Gentamicin high level - R Streptomycin high level - R			X

Appendix A. (Continued)

Organism or Organism Group	Antimicrobial Class/Subclass	Antimicrobial Agent(s) and 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 generally considered of epidemiological concern
Staphylococcus aureus	Penicillinase-stable penicillins	Oxacillin – R			X
	Cephems	Ceftaroline – SDD or R		X	
	Glycopeptides	Vancomycin – I[e]		X	
		Vancomycin – R	X		
	Lipoglycopeptides	Dalbavancin – NS Oritavancin – NS Telavancin – NS	X		
	Lipopeptides	Daptomycin – NS		X	
	Streptogramins	Quinupristin-dalfopristin (MSSA only) – I or R		X	
	Oxazolidinones	Linezolid – R Tedizolid – I or R		X	
	Pleuromutilins	**Lefamulin – NS**	**X**		
Staphylococcus spp. other than *S. aureus*	Glycopeptides	Vancomycin – I or R[f]		X	
	Lipopeptides	Daptomycin – NS		X	
	Oxazolidinones	Linezolid – R		X	

Appendix A. (Continued)

Organism or Organism Group	Antimicrobial Class/Subclass	Antimicrobial Agent(s) and 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 generally considered of epidemiological concern
Streptococcus pneumoniae	Penicillins	Amoxicillin or penicillin (nonmeningitis) – R			X
	Cephems	Cephalosporin III/IV (nonmeningitis) – R			X
		Ceftaroline (nonmeningitis) – NS	X		
	Carbapenems	Any carbapenem – I, R, or NS		X	
	Glycopeptides	Vancomycin – NS	X		
	Fluoroquinolones	Any fluoroquinolone – I or R		X	
	Streptogramins	Quinupristin-dalfopristin – I or R		X	
	Ansamycins	Rifampin – I or R		X	
	Oxazolidinones	Linezolid – NS	X		
	Pleuromutilins	**Lefamulin – NS**	**X**		
Streptococcus, β-hemolytic group	Penicillins	Ampicillin or penicillin – NS	X		
	Cephems	Cephalosporin III/IV – NS Ceftaroline – NS	X		
	Carbapenems	Any carbapenem – NS	X		
	Glycopeptides	Vancomycin – NS	X		
	Lipoglycopeptides	Dalbavancin – NS Oritavancin – NS Telavancin – NS	X X X		
	Lipopeptides	Daptomycin – NS	X		
	Streptogramins	Quinupristin-dalfopristin (*S. pyogenes* only) – I or R		X	
	Oxazolidinones	Linezolid – NS Tedizolid – NS	X X		

Appendix A. (Continued)

Organism or Organism Group	Antimicrobial Class/Subclass	Antimicrobial Agent(s) and 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 generally considered of epidemiological concern
Streptococcus, viridans group	Carbapenems	Any carbapenem – NS	X		
	Glycopeptides	Vancomycin – NS	X		
	Lipoglycopeptides	Dalbavancin (*S. anginosus* group only) – NS	X		
		Oritavancin – NS	X		
		Telavancin – NS	X		
	Streptogramins	Quinupristin-dalfopristin – I or R	X		
	Oxazolidinones	Linezolid – NS	X		
		Tedizolid – NS	X		
Neisseria meningitidis	Penicillins	Ampicillin or penicillin – I		X	
		Ampicillin or penicillin – R	X		
	Cephems	Cephalosporin III– NS	X		
	Carbapenems	Meropenem – NS	X		
	Macrolides	Azithromycin – NS		X	
	Tetracyclines	Minocycline – NS		X	
	Fluoroquinolones	Any fluoroquinolone – I or R		X	
	Phenicols	Chloramphenicol – I or R		X	
	Ansamycins	Rifampin – I or R		X	
Bacteroides spp. and *Parabacteroides* spp.	Carbapenems	Any carbapenem – I or R		X	
	Nitroimidazoles	Metronidazole – I or R		X	

Abbreviations: I, intermediate; ID, identification; mCIM, modified carbapenem inactivation method; MIC, minimal inhibitory concentration; MSSA, methicillin (oxacillin)-susceptible *Staphylococcus aureus;* NS, nonsusceptible; NWT, non-wild-type; R, resistant; SDD, susceptible-dose dependent.

Appendix A. (Continued)

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, MI-Cs in the intermediate or resistant category than those with meropenem or doripenem MICs. MICs for these agents may be elevated due to mechanisms other than carbapenemases among these organisms. A phenotypic test such as mCIM or CarbaNP may be used to identify carbapenemase-producing isolates (see Tables 3A and 3B).

c. Excludes organisms with intrinsic resistance to listed agents as described in Appendix B.

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. *S. aureus* isolates demonstrating vancomycin MICs 4 µg/mL may represent testing variation and need not be reported or submitted to public health department; *S. aureus* isolates demonstrating MICs > 4 µg/mL should be reported to the local public health department.

f. There are some *Staphylococcus* spp. other than *S. aureus* for which vancomycin MICs may test within the intermediate range (MIC 8–16 µg/mL). In contrast, vancomycin-resistant *Staphylococcus* spp. (MIC ≥ 32 µg/mL) are rare.

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.

NOTE 3: For strains yielding results in the "nonsusceptible" category, organism identification and antimicrobial susceptibility test results should be confirmed (see footnote "a").

NOTE 4: Information in boldface type is new or modified since the previous edition.

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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 prevention 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."

Appendix B. (Continued)

B1. Enterobacterales

Organism \ Antimicrobial Agent	Ampicillin	Amoxicillin-clavulanate	Ampicillin-sulbactam	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, *Citrobacter amalonaticus* group[a]	R			R									
Enterobacter cloacae complex[b]	R	R	R		R	R							
Escherichia coli	There is no intrinsic resistance to ß-lactams in this organism.												
Escherichia hermannii	R			R									
Hafnia alvei	R	R	R		R	R							
Klebsiella (formerly *Enterobacter*) *aerogenes*	R	R	R		R	R							
Klebsiella pneumoniae, *Klebsiella oxytoca*, *Klebsiella variicola*	R			R									
Morganella morganii	R	R			R		R	[c]		R	R	R	
Proteus mirabilis	There is no intrinsic resistance to penicillins and cephalosporins in this organism.							[c]	R	R	R	R	
Proteus penneri	R				R		R	[c]	R	R	R	R	
Proteus vulgaris	R				R		R	[c]	R	R	R	R	
Providencia rettgeri	R	R			R			[c]	R	R	R	R	
Providencia stuartii	R	R			R			[c]	R	R	R	R	[d]
Raoultella spp.[e]	R			R									

Appendix B. (Continued)

B1. Enterobacterales (Continued)

Organism \ Antimicrobial Agent	Ampicillin	Amoxicillin-clavulanate	Ampicillin-sulbactam	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.

<u>Footnotes</u>

a. *Citrobacter amalonaticus* group includes *C. amalonaticus, C. farmeri,* and *C. sedlakii.*

b. *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.

c. *Proteus* spp., *Providencia* spp., and *Morganella* spp. 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.

d. *P. stuartii* should be considered resistant to gentamicin, netilmicin, and tobramycin but not intrinsically resistant to amikacin.

e. *Raoultella* spp. includes *R. ornithinolytica, R. terrigena,* and *R. planticola.*

NOTE 1: Cephalosporins III, cefepime, aztreonam, ticarcillin-clavulanate, piperacillin-tazobactam, and the carbapenems are not listed, because there is no intrinsic resistance in Enterobacterales.

NOTE 2: Enterobacterales are also intrinsically resistant to clindamycin, daptomycin, fusidic acid, glycopeptides (vancomycin), lipoglycopeptides (oritavancin, teicoplanin, 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.

Appendix B. (Continued)

B2. Non-Enterobacterales

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				R						R			R				R		R	R
Burkholderia cepacia complex[a]	R	R	R	R	R	[a]	[a]	[a]		[a]	[a]	[a]		R	R	[a]		[a]			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: MIC, minimal inhibitory concentration; R, resistant.

Footnotes

a. *B. cepacia* complex isolates have chromosomal genes that must undergo mutational changes before expressing resistance. It is not known how often these mutations occur during growth. Intrinsic resistance implies the presence of resistance mechanisms in natural or wild-type strains that result in phenotypic resistance for all or nearly all strains. Environmental *B. cepacia* complex strains lacking mutations do not express resistance mechanisms, resulting in low MICs to many antimicrobial agents, whereas clinical strains that express resistance genes, such as those from cystic fibrosis patients, have high MIC values to these same antimicrobial agents. There is insufficient clinical evidence to confirm whether strains that test susceptible *in vitro,* despite the presence of resistance mechanisms, will respond *in vivo*. Therefore, intrinsic resistance to the footnoted antibiotics (listed as resistant in previous editions of M100) cannot be confirmed.

b. *S. maltophilia* is intrinsically resistant to tetracycline but not to doxycycline, minocycline, or tigecycline.

NOTE 1: 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), linezolid, macrolides (erythromycin, azithromycin, clarithromycin), quinupristin-dalfopristin, and rifampin.

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

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		

Abbreviations: MRS, methicillin (oxacillin) resistant staphylococci; R, resistant.

NOTE 1: These gram-positive bacteria are also intrinsically resistant to aztreonam, polymyxin B/colistin, and nalidixic acid.

NOTE 2: MRS, as defined by cefoxitin or oxacillin testing, as appropriate to the species, are considered resistant to other β-lactam agents, ie, penicillins, β-lactam combination agents, cephems with the exception of ceftaroline, 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.

Appendix B. (Continued)

B4. Enterococcus spp.

Organism \ Antimicrobial Agent	Cephalosporins	Vancomycin	Teicoplanin	Aminoglycosides	Clindamycin	Quinupristin-dalfopristin	Trimethoprim	Trimethoprim-sulfamethoxazole	Fusidic Acid
E. faecalis	R[a]			R[a]	R[a]	R	R	R[a]	R
E. faecium	R[a]			R[a]	R[a]		R	R[a]	R
E. gallinarum/E. casseliflavus	R[a]	R		R[a]	R[a]	R	R	R[a]	R

Abbreviation: R, resistant.

a. **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.

Appendix B. (Continued)

B5. Anaerobic Gram-Positive Bacilli

Organism \ Antimicrobial Agent	Vancomycin	Aminoglycosides
Clostridium and *Clostridioides* 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)	• ß-lactam combination agents	• ß-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.

Appendix C. (Continued)

QC Strains	Organism Characteristics	Disk Diffusion Tests	MIC Tests	Other Tests	Comments
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	
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]	• TEM-1	• β-lactam combination agents	• β-lactam combination agents		
E. coli NCTC 13353[a,b,2]	• CTX-M-15 (ESBL)	• ß-lactam combination agents	• ß-lactam combination agents		
E. coli AR Bank #0349[3]	• MCR-1			• Colistin broth disk elution • Colistin agar test	
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 ß-lactam agents
Klebsiella pneumoniae ATCC® 700603[a,b,1,4]	• SHV-18 (ESBL) • OXA-2 • Mutations in OMPK35 and OMPK37	• β-lactam combination agents	• β-lactam combination agents	• ESBL tests	

Appendix C. (Continued)

QC Strains	Organism Characteristics	Disk Diffusion Tests	MIC Tests	Other Tests	Comments
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-2146™	• NDM			• 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™
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 • ICR 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 • ICR MIC test • Penicillin zone-edge test	• Assess suitability of cation content in each batch/lot of MHB for daptomycin broth microdilution.

Appendix C. (Continued)

QC Strains	Organism Characteristics	Disk Diffusion Tests	MIC Tests	Other Tests	Comments
S. aureus ATCC® 43300	• *mecA* positive	• Cefoxitin disk diffusion testing	• Cefoxitin MIC testing	• Oxacillin salt agar	
S. aureus ATCC® BAA-976™	• *msrA*-mediated macrolide-only resistance			• ICR MIC test and disk approximation test (D-zone test)	
S. aureus ATCC® BAA-977™	• Inducible *ermA*-mediated macrolide resistance			• ICR MIC test and disk approximation test (D-zone test)	
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*	• ICR 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; ICR, inducible clindamycin resistance; MHA, Mueller-Hinton agar; MHB, Mueller-Hinton broth; MIC, minimal inhibitory concentration; NCTC, National Collection of Type Cultures; QC, quality control.

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 ß-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 ß-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 ß-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.

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[5] and M07.[6] 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.

References for Appendix C

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

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

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Appendix D. Anaerobe Cumulative Antibiogram[1]

NOTE: Isolates collected from selected US hospitals from 1 January 2013 to 31 December 2016.[a]

D1. *Bacteroides* spp. and *Parabacteroides* spp.

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)[b]		%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[c]	84[c]	16[c]	49	100	0	236	95	1
B. vulgatus	20[c]	45[c]	15[c]	168	92	0	153	73	14	—	—	—	35	97	0	171	96	4
B. uniformis	19[c]	84[c]	0[c]	78	96	0	72	85	10	—	—	—	19[c]	100[c]	0[c]	93	100	0
Parabacteroides distasonis	27[c]	59[c]	19[c]	92	95	1	82	29	43	—	—	—	26[c]	100[c]	0	119	97	2

Appendix D. (Continued)

D1. *Bacteroides* spp. and *Parabacteroides* spp. (Continued)

Anaerobic Organisms	Number of Strains	Clindamycin		Number of Strains	Moxifloxacin		Number of Strains	Metronidazole	
Percent susceptible (%S) and percent resistant (%R)[b]		%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[c]	31[c]	45[c]	186	100	0
B. uniformis	87	45	48	25[c]	48[c]	40[c]	89	100	0
Parabacteroides distasonis	108	43	44	37	62	35	118	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. Intermediate category is not shown but can be derived by subtraction of %S and %R for each antimicrobial agent from %100.

c. Calculated from fewer than the CLSI document M39[1] recommendation of 30 isolates.

Reference for D1

1 CLSI. *Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data; Approved Guideline—Fourth Edition.* CLSI document M39-A4. Clinical and Laboratory Standards Institute; 2014.

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* spp. and *Parabacteroides* spp.

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)[b]		%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[c]	97[c]	3[c]	63	100	0	29[c]	100	0	92	98	0	63	100	0
Fusobacterium spp.	20[c]	100[c]	0[c]	55	96	2	75	95	4	20[c]	100[c]	0[c]	–[d]	–[d]	–[d]
Anaerobic gram-positive cocci[e]	–[d]	–[d]	–[d]	1853	99	1	134	99	0	1647	100	0	1647	100	0
Cutibacterium (formerly *Propionibacterium*) *acnes*[f]	–[d]	–[d]	–[d]	18[c]	100[c]	0[c]	17[c]	94[c]	0[d]	–[d]	–[d]	–[d]	–[d]	–[d]	–[d]
Clostridium perfringens	15[c]	100[c]	0	410	100	0	23[c]	100[c]	0[c]	417	100	0	402	90	4
Clostridioides (formerly *Clostridium*) *difficile*[g]	76	99	0	542	93	0	480	69	4	609	99	0	533	6	37
Other *Clostridium* spp.	–[d]	–[d]	–[d]	439	94	1	71	99	0	390	100	0	390	69	13

Appendix D. (Continued)

D2. Anaerobic Organisms Other Than *Bacteroides* spp. and *Parabacteroides* spp. (Continued)

Anaerobic Organisms	Number of Strains	Clindamycin		Number of Strains	Moxifloxacin		Number of Strains	Metronidazole	
Percent susceptible (%S) and percent resistant (%R)[b]		%S	%R		%S	%R		%S	%R
Breakpoints in µg/mL		≤ 2	≥ 8		≤ 2	≥ 8		≤ 8	≥ 32
Prevotella spp.	29[c]	69[c]	28[c]	92	66	25	92	99	0
Fusobacterium spp.	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*[f]	17[c]	53[c]	35[c]	114	95	4	18[c]	0[c]	100[c]
C. perfringens	425	83	12	23[c]	83[c]	9[c]	425	100	0
Clostridioides (formerly *Clostridium*) *difficile*[g]	1013	32	38	480	74	25	1343	100	0
Other *Clostridium* spp.	461	67	25	71	62	35	461	100	0

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. Intermediate category is not shown but can be derived by subtraction of %S and %R for each antimicrobial agent from %100.

c. Calculated from fewer than the CLSI document M39[1] recommendation of 30 isolates.

d. A dash (–) indicates that data were not available.

e. Anaerobic gram-positive cocci include *Peptococcus*, *Peptostreptococcus*, *Finegoldia*, *Peptoniphilus*, and *Anaerococcus* species.

f. 80 isolates of *Cutibacterium* (formerly *Propionibacterium*) *acnes* from two of the sites generated MIC values for rifampin ≤ 0.03 µg/mL using the agar dilution method. There are no interpretive breakpoints for this organism/antimicrobial agent combination.

g. *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.

Reference for D2

[1] CLSI. *Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data; Approved Guideline—Fourth Edition*. CLSI document M39-A4. 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. Enterobacterales				
Azithromycin (*Salmonella enterica* ser. Typhi and *Shigella* spp.)	≤16 µg/mL	500 mg administered daily	N/A	
Aztreonam	≤4 µg/mL	1 g administered every 8 h	N/A	
Cefazolin	≤2 µg/mL	2 g administered every 8 h	N/A	
Ceftaroline	≤0.5 µg/mL	600 mg administered every 12 h	N/A	
Cefepime	≤2 µg/mL	1 g administered every 12 h	4 µg/mL	1 g administered every 8 h or 2 g administered every 12 h
			8 µg/mL or zone diameter: 19-24 mm	2 g administered 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.)
Cefiderocol	≤4 µg/mL	2 g every 8 h administered over 3 h	N/A	
Cefotaxime	≤1 µg/mL	1 g administered every 8 h	N/A	
Ceftriaxone	≤1 µg/mL	1 g administered every 24 h	N/A	
Cefoxitin	≤8 µg/mL	8 g per day (eg, 2 g administered every 6 h)	N/A	
Cefuroxime	≤8 µg/mL	1.5 g administered every 8 h	N/A	
Ceftazidime	≤4 µg/mL	1 g administered every 8 h	N/A	
Ceftazidime-avibactam	≤8/4 µg/mL	2.5 g (2 g ceftazidime + 0.5 g avibactam) every 8 h administered over 2 h	N/A	
Ceftizoxime	≤1 µg/mL	1 g administered every 12 h	N/A	
Ceftolozane-tazobactam	≤2/4 µg/mL	1.5 g administered every 8 h	N/A	
Ciprofloxacin	≤0.25 µg/mL	400 mg IV or 500 mg orally administered every 12 h	N/A	
Colistin or polymyxin B	≤2 µg/mL[a]	See International Consensus Guidelines[1] for dosage recommendations.	N/A	
Doripenem	≤1 µg/mL	500 mg administered every 8 h	N/A	
Ertapenem	≤0.5 µg/mL	1 g administered every 24 h	N/A	

Appendix E. (Continued)

Antimicrobial Agent	Breakpoints and Interpretive Categories			
	Susceptible		SDD	
	MIC	Dose	MIC	Dose
Table 2A. Enterobacterales (Continued)				
Imipenem	≤ 1 μg/mL	500 mg administered every 6 h or 1 g every 8 h	N/A	Imipenem
Imipenem-relebactam	**≤ 1/4 μg/mL**	**1.25 g administered every 6 h**	**N/A**	**Imipenem-relebactam**
Levofloxacin	≤ 0.5 μg/mL	750 mg administered every 24 h	N/A	Levofloxacin
Meropenem	≤ 1 μg/mL	1 g administered every 8 h	N/A	Meropenem
Meropenem-vaborbactam	≤ 4/8 μg/mL	4 g (2 g meropenem + 2 g vaborbactam) every 8 h administered over 3 h	N/A	Meropenem-vaborbactam
Table 2B-1. *Pseudomonas aeruginosa*				
Aztreonam	≤ 8 μg/mL	1 g administered every 6 h or 2 g every 8 h	N/A	
Cefepime	≤ 8 μg/mL	1 g administered every 8 h or 2 g every 12 h	N/A	
Cefiderocol	≤ 4 μg/mL	2 g every 8 h administered over 3 h	N/A	
Ceftazidime	≤ 8 μg/mL	1 g administered 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) administered every 8 h over 2 h	N/A	
Ceftolozane-tazobactam	**≥ 4/4**	**1.5 g administered every 8 h**	**N/A**	
Ciprofloxacin	≤ 0.5 μg/mL	400 mg IV administered every 8h	N/A	
Colistin or polymyxin B	≤ 2 μg/mL[a]	See International Consensus Guidelines[1] for dosage recommendations	N/A	
Doripenem	≤ 2 μg/mL	500 mg administered every 8 h	N/A	
Imipenem	≤ 2 μg/mL	1 g administered every 8 h or 500 mg every 6 h	N/A	
Imipenem-relebactam	**≤ 2/4 μg/mL**	**1.25 g administered every 6 h**	**N/A**	
Levofloxacin	≤ 1 μg/mL	750 mg administered every 24 h	N/A	
Meropenem	≤ 2 μg/mL	1 g administered every 8 h	N/A	
Piperacillin	≤ 16 μg/mL	3 g administered every 6 h	N/A	
Piperacillin-tazobactam	≤ 16/4 μg/mL	3 g administered every 6 h	N/A	
Ticarcillin	≤ 16 μg/mL	3 g administered every 6 h	N/A	
Ticarcillin-clavulanate	≤ 16/2 μg/mL	3 g administered every 6 h	N/A	
Table 2B-2. *Acinetobacter* spp.				
Cefiderocol	≤ 4 μg/mL	2 g every 8 h administered over 3 h	N/A	
Colistin or polymyxin B	≤ 2 μg/mL[a]	See International Consensus Guidelines[1] for dosage recommendations	N/A	
Doripenem	≤ 2 μg/mL	500 mg administered every 8 h	N/A	
Imipenem	≤ 2 μg/mL	500 mg administered every 6 h	N/A	
Meropenem	≤ 2 μg/mL	1 g administered every 8 h or 500 mg every 6 h	N/A	
Table 2B-4. *Stenotrophomonas maltophilia*				
Cefiderocol	≤ 4 μg/mL	2 g every 8 h administered over 3 h	N/A	

Appendix E. (Continued)

Antimicrobial Agent	Breakpoints and Interpretive Categories			
	Susceptible		SDD	
	MIC	Dose	MIC	Dose
Table 2C. *Staphylococcus* spp				
Ceftaroline (*S. aureus* only)	≤ 1 µg/mL	600 mg administered every 12 h	2-4 µg/mL	600 mg every 8 h administered over 2 h **NOTE:** For *S. aureus* only.
Dalbavancin	≤ 0.25 µg/mL	1500 mg (single dose) IV administered over 30 minutes or 1000 mg (two does) followed one week later by 500 mg IV administered over 30 minutes (adult patients with creatinine clearance ≥ 30 mL/minute)	N/A	
Lefamulin (*S. aureus* only)	**≤ 0.25 µg/mL**	**150 mg IV or 600 mg orally administered every 12 h**	**N/A**	
Oritavancin	≤ 0.12 µg/mL	1200 mg single IV dose	N/A	
Tedizolid	≤ 0.5 µg/mL	200 mg administered every 24 h	N/A	
Telavancin	≤ 0.12 µg/mL	10 mg/kg administered every 24 h	N/A	
Table 2D. *Enterococcus* spp.				
Dalbavancin	≤ 0.25 µg/mL	1500 mg (single dose) IV administered over 30 minutes or 1000 mg (two does) followed one week later by 500 mg IV administered over 30 minutes (adult patients with creatinine clearance ≥ 30 mL/minute).	N/A	
Daptomycin *E. faecium* only	N/A	N/A	≤ 4 µg/mL	8-12 mg/kg administered every 24 h
Daptomycin *Enterococcus* spp. other than *E. faecium*	≤ 2 µg/mL	6 mg/kg administered every 24 h	N/A	
Oritavancin	≤ 0.12 µg/mL	1200 mg single IV dose	N/A	
Tedizolid	≤ 0.5 µg/mL	200 mg administered every 24 h	N/A	
Telavancin	≤ 0.25 µg/mL	10 mg/kg administered every 24 h	N/A	
Table 2E. *Haemophilus influenzae* and *Haemophilus parainfluenzae*				
Ceftaroline (***H. influenzae* only)**	≤ 0.5 µg/mL	600 mg administered every 12 h	N/A	
Ceftolozane-tazobactam (*H. influenzae* only)	**≤ 0.5/4 µg/mL**	**1.5 g administered every 8 h**	**N/A**	
Lefamulin (*H. influenzae* only)	**≤ 2 µg/mL**	**150 mg IV or 600 mg orally administered every 12 h**	**N/A**	
Table 2F. *Neisseria gonorrhoeae*				
Azithromycin	**≤ 1 µg/mL**	**1 g single dose**		
Table 2G. *Streptococcus pneumoniae*				
Ceftaroline (nonmeningitis)	≤ 0.5 µg/mL	600 mg administered every 12 h	N/A	
Lefamulin	**≤ 0.25 µg/mL**	**150 mg IV or 600 mg orally administered every 12 h**	**N/A**	
Penicillin (nonmeningitis)	≤ 2 µg/mL	2 million units administered every 4 h (12 million units per day)	N/A	
Penicillin parenteral (meningitis)	≤ 0.06 µg/mL	3 million units administered every 4 h	N/A	

Appendix E. (Continued)

Antimicrobial Agent	Breakpoints and Interpretive Categories			
	Susceptible		SDD	
	MIC	Dose	MIC	Dose
Table 2H-1. *Streptococcus* spp. β-Hemolytic Group				
Ceftaroline	≤ 0.5 µg/mL	600 mg administered every 12 h	N/A	
Dalbavancin	≤ 0.25 µg/mL	1500 mg (single dose) IV administered over 30 minutes or 1000 mg (two doses) followed one week later by 500 mg IV administered 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 administered every 24 h	N/A	
Telavancin	≤ 0.12 µg/mL	10 mg/kg administered every 24 h	N/A	
Table 2H-2. *Streptococcus* spp. Viridans Group				
Dalbavancin	≤ 0.25 µg/mL	1500 mg (single dose) IV administered over 30 minutes or 1000 mg (two doses) followed one week later by 500 mg IV administered 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 administered every 24 h	N/A	
Telavancin	≤ 0.06 µg/mL	10 mg/kg administered every 24 h	N/A	
Table 2J. Anaerobes				
Imipenem-relebactam	**≤ 4/4 µg/mL**	**1.25 g administered every 6 h**	**N/A**	

Abbreviations: IV, intravenous; MIC, minimal inhibitory concentration; N/A, not applicable; SDD, susceptible-dose dependent.

Footnote

a. MIC ≤ 2 µg/mL for colistin and polymyxin B corresponds to intermediate category.

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

Reference for Appendix E

[1] Tsuji BT, Pogue JM, Zavascki AP, et al. International consensus guidelines for the optimal use of the polymyxins: endorsed by the American College of Clinical Pharmacy (ACCP), European Society of Clinical Microbiology and Infectious Diseases (ESCMID), Infectious Diseases Society of America (IDSA), International Society for Anti-Infective Pharmacology (ISAP), Society of Critical Care Medicine (SCCM), and Society of Infectious Diseases Pharmacists (SIDP). *Pharmacotherapy*. 2019;39(1):10-39.

Appendix F. Susceptible-Dose Dependent Interpretive Category

Abbreviations for Appendix F

AST	antimicrobial susceptibility testing
FDA	US Food and Drug Administration
MIC	minimal inhibitory concentration
QC	quality control
SDD	susceptible-dose dependent

Susceptible-dose dependent (SDD) is recommended instead of "intermediate" for several drug and organism combinations for which there are multiple approved or routinely used dosing options:

- Enterobacterales: cefepime
- *Staphylococcus aureus*: ceftaroline
- *Enterococcus faecium*: daptomycin

SDD highlights the option of using higher doses or alternative dosing regimens by which to achieve a higher dose exposure for the treatment of infections caused by isolates when the minimal inhibitory concentration (MIC) or the zone diameter is in the SDD range.

What does SDD mean?

SDD is a category defined by a breakpoint that implies that susceptibility of an isolate depends on the dosing regimen that is used in the patient. To achieve levels that are likely to be clinically effective against isolates for which the susceptibility testing results (either 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 that achieved with the dose that was used to establish the susceptible breakpoint. Consideration should be given to the maximum, literature-supported dosage regimens, because higher exposure gives the highest probability of adequate coverage of an SDD isolate. Appendix E lists the doses used when establishing SDD categories. 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 supported by the literature, widely used clinically, and/or approved and for which sufficient data to justify the designation exist and have been reviewed. When the intermediate category is used, its definition remains unchanged.

Appendix F. (Continued)

Why is SDD being used now?

- 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 conveys what we know—a higher dose can be considered for isolates with MICs (or zones of inhibition) that fall in this interpretive category.
- SDD is already well established for use in antifungal susceptibility testing.
- 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.

How should this change be implemented?

- Meet with the appropriate practitioners at your institution (eg, members of the antimicrobial stewardship team, infectious diseases staff, pathology group, pharmacy) to explain SDD and determine a plan for implementation, if appropriate.
- Talk to the manufacturer of your antimicrobial susceptibility testing (AST) device to determine how to implement reporting SDD on your device.
  - **NOTE:** Because the US Food and Drug Administration (FDA) does not yet recognize the SDD interpretive category and commercial manufacturers must use FDA breakpoints, the manufacturer cannot adopt the CLSI SDD 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 dose ("D") when MICs or zone diameters are in the SDD range. Some laboratory information systems may handle only a single character and use of "D" for "dose" may be appropriate. Ideally, this could be translated to SDD on the final patient report. Regardless of approach, 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.

Appendix F. (Continued)

Additional Questions and Answers:

1. Q: Does CLSI recommend a comment to be reported with the new SDD 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 [dose] (refer to Appendix E). The interpretive criterion for SDD is based on dosage regimens that result in higher antimicrobial exposure, either higher doses or more frequent doses, or both."

2. Q: Will all intermediate ranges become SDD?

   A: No, the SDD category will be implemented for drug and 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 and organism combinations for which multiple dosing options exist and have been well studied.

4. Q: How do we perform a verification study before implementing the new breakpoints on our AST device?

   A: Guidelines for performance of such a verification study are available (see CLSI document M52[1]).[2]

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 while considering all other clinical and physiological parameters for that patient.

6. Q: Is any special QC needed once the SDD breakpoints are implemented?

   A: No, currently recommended routine QC is sufficient.

7. 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)

8. Q: If we can implement the revised 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 prevention practitioners.

9. Q: If we can implement the revised breakpoints but cannot facilitate reporting of SDD, can we report an MIC or zone diameter without an interpretation?

   A: A zone diameter should never be reported without an interpretation because there is a high risk of misinterpretation of this value, which 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 8, above.

10. 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. *Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems*. 1st ed. CLSI guideline M52. Clinical and Laboratory Standards Institute; 2015.

2 Patel J, Sharp S, Novak-Weekley S. Verification of antimicrobial susceptibility testing methods: a practical approach. *Clin Microbiol Newslett*. 2013;35(13):103-109.

Appendix G. Epidemiological Cutoff Values

Abbreviations for Appendix G

ECV	epidemiological cutoff value
MIC	minimal inhibitory concentration
NWT	non-wild-type
WT	wild-type

G1 CLSI Epidemiological Cutoff Value Additions/Revisions Since 2015

Antimicrobial Agent	Date of Addition/Revision (M100 edition)	Comments
Anaerobes		
Vancomycin	January 2015 (M100-S25)	For use with *Cutibacterium* (formerly *Propionibacterium*) *acnes*.

G2 Defining Epidemiological Cutoff Values

G2.1 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:

Interpretive Category	ECVs	
	MIC, µg/mL	Zone Diameter, mm
Wild-type	≤ 4	≥ 20
Non-wild-type	≥ 8	≤ 19

- **wild-type (WT)** - an interpretive category defined by an ECV that describes the microbial population with no phenotypically detectable mechanisms of resistance or reduced susceptibility for the antimicrobial (antifungal) agent being evaluated.

- **non-wild-type (NWT) - an interpretive category defined by an ECV that describes the microbial population with phenotypically detectable mechanisms of resistance and reduced susceptibility for the antimicrobial (antifungal) agent being evaluated.**

Appendix G. (Continued)

G2.2 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.

G2.3 Establishing Epidemiological Cutoff Values

ECVs are determined by collecting and merging MIC distribution data obtained by testing microbes 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. 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.

Several conditions must be fulfilled to generate reliable ECVs. The most important are:

- An ECV can be determined only 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.

Appendix G. (Continued)

- 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]
  - Establishment 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. In such cases, the ECV may need to be reassessed.

G2.4 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.

Appendix G. (Continued)

References for G2

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

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

3 ISO. *Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices – Part 1: Broth micro-dilution 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; 2019.

4 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. 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.

G3 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 Table G1 are applicable only to the species indicated. Currently, there are insufficient data to support their use with other species.

Table G1. 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.

Appendix G. (Continued)

References for Table G1

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.

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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Appendix H. Using Molecular Assays for Resistance Detection

Abbreviations for Appendix H

AST	antimicrobial susceptibility testing
ESBL	extended-spectrum ß-lactamase
MIC	minimal inhibitory concentration
MRSA	methicillin (oxacillin)-resistant Staphylococcus aureus
N/A	not applicable
PBP2a	penicillin-binding protein 2a
VRE	vancomycin-resistant enterococci

Antimicrobial resistance and susceptibility are complex, and current *in vitro* methods have been developed to predict a microorganism's response to antibacterial therapy *in vivo*. Standardized phenotypic methods have evolved over many decades, but faster and potentially more reliable nucleic acid– and protein-based methods have been recently developed to detect antimicrobial resistance. The current challenge for medical laboratories is to integrate molecular assays for antimicrobial resistance determinants with conventional antimicrobial susceptibility testing (AST) procedures, sometimes despite an incomplete understanding of test limitations.

The tables in this appendix provide a practical approach for testing and reporting results among medical laboratories that routinely use molecular techniques (with or without a phenotypic test) for detecting antimicrobial resistance. Antimicrobial resistance is genetically complex and based on available data. Molecular methods are often used as a screening tool (eg, methicillin [oxacillin]-resistant *Staphylococcus aureus* [MRSA] from nasal swabs) or as a rapid adjunct to traditional phenotypic methods (eg, KPC from instrument-flagged blood culture bottles). Interpretation necessitates critical thinking and an understanding of the dynamics between detecting "resistance" determinants and testing phenotypic "susceptibility." Detecting a resistance marker does not necessarily predict therapeutic failure of antimicrobial agents. The gene may be nonfunctional or expressed at clinically insignificant levels. Conversely, the absence of the genetic marker does not necessarily indicate susceptibility, because technical issues may interfere with detection (eg, inhibition of amplification, emergence of genetic variants). In some cases, a molecular approach may be superior to traditional phenotypic methods, such as in the case of low *in vitro* expression, heteroresistance, or poor growth masking higher minimal inhibitory concentrations (MICs). Overall, laboratorians should attempt to apply a consistent approach to molecular-based methods and aim to resolve discordant results with repeat or supplementary testing, by referral to a reference laboratory or by reporting both results in accordance with institutional policies.

As understanding of the molecular mechanisms of antimicrobial resistance continues to develop, more sophisticated approaches to molecular detection of antimicrobial resistance in the medical microbiology laboratory will undoubtedly emerge. The following tables will be updated as needed to ensure the provision of relevant guidance as methods evolve.

Appendix H. (Continued)

Table H1. Strategies for Reporting Methicillin (Oxacillin) Results When Using Molecular and Phenotypic AST Methods for *S. aureus*

Indication	Target(s)	Method	Specimen Type	Results: Genotype or Predicted Phenotype	Results: Observed Colony Phenotype (if tested)	Suggestions for Resolution	Consider reporting as[a]:	Comments[b]
Detecting methicillin (oxacillin) resistance in *S. aureus*	PBP2a	Latex agglutination, immuno-chromatography	Colony	PBP2a positive	Cefoxitin R	N/A	Methicillin (oxacillin) R	1
				PBP2a negative	Cefoxitin S	N/A	Methicillin (oxacillin) S	1
				PBP2a positive	Cefoxitin S	Confirm isolate identification, repeat latex agglutination and AST, and consider *mecA* colony NAAT, if available.	If discrepancy is not resolved by suggested testing, report as methicillin (oxacillin) R.	1-2
				PBP2a negative	Cefoxitin R	Confirm isolate identification, repeat latex agglutination and AST, and consider *mecA* colony NAAT, if available.	If discrepancy is not resolved by suggested testing, report as methicillin (oxacillin) R.	1
	mecA	NAAT, microarray hybridization, ISH	Colony, blood culture broth, surveillance specimen	*mecA* detected	Cefoxitin R	N/A	If tested, report phenotypic result as found (methicillin [oxacillin] R) and consider reporting molecular result per institutional protocol.	3-6
				mecA not detected	Cefoxitin S	N/A	If tested, report phenotypic result as found (methicillin [oxacillin] S) and consider reporting molecular result per institutional protocol.	3-6
				mecA detected	Cefoxitin S	Confirm isolate identification, repeat AST, and repeat or perform *mecA* colony NAAT, if available. If mixed specimen, test isolates individually.	If discrepancy is not resolved by suggested testing, report as methicillin (oxacillin) R.	2-5, 8-9
				mecA not detected	Cefoxitin R	Confirm isolate identification, repeat AST, and repeat or perform *mecA* colony NAAT, if available. If mixed specimen, test isolates individually.	If discrepancy is not resolved by suggested testing, report as methicillin (oxacillin) R.	3, 7

Appendix H. (Continued)

Table H1. (Continued)

Indication	Target(s)	Method	Specimen Type	Results		Suggestions for Resolution	Consider reporting as[a]:	Comments[b]
				Genotype or Predicted Phenotype	Observed Colony Phenotype (if tested)			
Detecting methicillin (oxacillin) resistance in *S. aureus* (Continued)	SCC*mec*-*orfX* functional regions only	NAAT	Blood culture broth, surveillance specimen	SCC*mec* detected	Cefoxitin R	N/A	If tested, report phenotypic result as found (methicillin [oxacillin] R) and consider reporting molecular result per institutional protocol.	3-6
				SCC*mec* not detected	Cefoxitin S	N/A	If tested, report phenotypic result as found (methicillin [oxacillin] S) and consider reporting molecular result per institutional protocol.	3-6
				SCC*mec* detected	Cefoxitin S	Confirm isolate identification, repeat AST and consider *mecA* colony NAAT, if available. If mixed culture, test isolates individually.	If discrepancy is not resolved by suggested testing, report as methicillin (oxacillin) R.	2, 10
				SCC*mec* not detected	Cefoxitin R	Confirm isolate identification, repeat AST and consider *mecA* colony NAAT, if available. If mixed culture, test isolates individually.	If discrepancy is not resolved by suggested testing, report as methicillin (oxacillin) R.	7, 12

Appendix H. (Continued)

Table H1. (Continued)

Indication	Target(s)	Method	Specimen Type	Results: Genotype or Predicted Phenotype	Results: Observed Colony Phenotype (if tested)	Suggestions for Resolution	Consider reporting as[a]:	Comments[b]
Detection of methicillin (oxacillin) resistance in *S. aureus* (Continued)	SCC*mec-orfX* junctional regions and *mecA* and/or other targets	NAAT	Blood culture broth, surveillance specimen	SCC*mec* AND *mecA* or other target detected	Cefoxitin R	N/A	If tested, report phenotypic result as found (methicillin [oxacillin] R) and consider reporting molecular result per institutional protocol.	3-6
				SCC*mec* AND *mecA* or other target not detected	Cefoxitin S	N/A	If tested, report phenotypic result as found (methicillin [oxacillin] S) and consider reporting molecular result per institutional protocol.	3-6
				SCC*mec* AND *mecA* or other target detected	Cefoxitin S	Confirm isolate identification, repeat AST and consider *mecA* colony NAAT, if available. If mixed culture, test isolates individually.	If discrepancy is not resolved by suggested testing, report as methicillin (oxacillin) R.	2
				SCC*mec* AND *mecA* or other target not detected	Cefoxitin R	Confirm isolate identification, repeat AST and consider *mecA* colony NAAT, if available. If mixed culture, test isolates individually.	If discrepancy is not resolved by suggested testing, report as methicillin (oxacillin) R.	3, 11

Abbreviations: AST, antimicrobial susceptibility testing; ISH, *in situ* hybridization; MSSA, methicillin (oxacillin)-susceptible *Staphylococcus aureus;* MRSA, methicillin (oxacillin)-resistant *S. aureus*; N/A, not applicable; NAAT, nucleic acid amplification test; PBP2a, penicillin-binding protein 2a; PCR, polymerase chain reaction; R, resistant; S, susceptible.

Appendix H. (Continued)

Table H1. (Continued)

Comments

(1) False-positive and false-negative PBP2a latex bead agglutination results have been observed.[1]

(2) Rare *mecA*-positive *S. aureus* isolates will test susceptible to cefoxitin.[2,3]

(3) *mecC* or *mecA* variant gene-mediated methicillin (oxacillin) resistance may not be detected by *mecA* PCR.[4,5]

(4) The simultaneous presence of *mecA*-positive *Staphylococcus* spp. (other than *S. aureus*) and MSSA may result in false-positive MRSA molecular results.[6,7]

(5) Strains harboring unstable SCC*mec* insertions may lose *mecA* during culture.[8]

(6) Compared with culture, the sensitivity of molecular methods may be higher, while the specificity may be lower.

(7) Occasional false-negative *mecA* results have been reported for direct blood culture molecular assays.[9]

(8) For ISH assays with a cefoxitin induction step, false-positive *mecA* results should be rare.[10]

(9) In polymicrobial cultures, the presence of *mecA* cannot be attributed to a specific isolate.

(10) Strains harboring an SCC*mec* remnant lacking the *mecA* gene (*mecA* dropout) or mutant *mecA* allele may test positive in assays that target only SCC*mec*-*orfX* junctional regions. Laboratories using molecular tests that detect only SCC*mec*-*orfX* junctional region targets may consider adding a disclaimer to the report stating the proportion of false-positive results related to *mecA* dropouts observed in isolates from the patient population served.[11]

(11) Multiple SCC*mec* types exist; depending on the design of the assay, some SCC*mec* variants may not be detected.[12]

Footnotes

a. Isolates that test as methicillin resistant are also oxacillin resistant, and the term "methicillin R" is synonymous with "oxacillin R."

b. In addition to the specific possibilities listed in the comments, genotype and/or phenotype discrepancies could arise as a consequence of suboptimal sampling, mixed cultures, emergence of new genotypes or mutations, and/or wild-type reversions of resistance targets.

Appendix H. (Continued)

Table H1. (Continued)

References for Table H1

1 Bressler AM, Williams T. Culler EE, et al. Correlation of penicillin binding protein 2a detection with oxacillin resistance in *Staphylococcus aureus* and discovery of a novel penicillin binding protein 2a mutation. *J Clin Microbiol*. 2005;43(9):4541-4544.

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3 Swenson JM, Tenover FC; Cefoxitin Disk Study Group. Results of disk diffusion testing with cefoxitin correlate with presence of *mecA* in *Staphylococcus* spp. *J Clin Microbiol*. 2005;43(8):3818-3823.

4 Shore AC, Deasy EC, Slickers P, et al. Detection of staphylococcal cassette chromosome mec type XI carrying highly divergent *mecA*, *mecI*, *mecR1*, *blaZ*, and *ccr* genes in human clinical isolates of clonal complex 130 methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother*. 2011;55(8):3765-3773.

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7 Shore AC, Rossney AS, O'Connell B, et al. Detection of staphylococcal cassette chromosome *mec*-associated DNA segments in multiresistant methicillin-susceptible *Staphylococcus aureus* (MSSA) and identification of *Staphylococcus epidermidis* ccrAB4 in both methicillin-resistant *S. aureus* and MSSA. *Antimicrob Agents Chemother*. 2008;52(12):4407-4419.

8 Wong H, Louie L, Lo RY, Simor AE. Characterization of *Staphylococcus aureus* isolates with a partial or complete absence of staphylococcal cassette chromosome elements. *J Clin Microbiol*. 2010;48(10):3525-3531.

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10 Salimnia H, Fairfax MR, Lephart P, et al. An international, prospective, multicenter evaluation of the combination of AdvanDx *Staphylococcus* QuickFISH BC with mecA XpressFISH for detection of methicillin-resistant *Staphylococcus aureus* isolates from positive blood cultures. *J Clin Microbiol*. 2014;52(11):3928-3932.

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12 Deurenberg RH, Vink C, Kalenic S, Friedrich AW, Bruggeman CA, Stobberingh EE. The molecular evolution of methicillin-resistant *Staphylococcus aureus*. *Clin Microbiol Infect*. 2007;13(3):222-235.

Appendix H. (Continued)

Table H2. Strategies for Reporting Vancomycin Results When Using Molecular and Phenotypic Antimicrobial Susceptibility Testing Methods for *Enterococcus* spp.

Indication	Target(s)	Method	Specimen Type	Results: Genotype or Predicted Phenotype	Results: Observed Phenotype (if tested)	Suggestions for Resolution	Report as:	Comments[a]
Detection of vancomycin-resistant enterococci	*vanA* *vanB*	NAAT or array hybridization technology	Blood culture broth or surveillance cultures	*vanA* and/or *vanB* detected	Vancomycin R	N/A	Report phenotypic result as found (if available); consider reporting presence of molecular target per institutional protocol.	1-3
				vanA and/or *vanB* not detected	Vancomycin S	N/A	Report phenotypic result as found (if available); consider reporting presence of molecular target per institutional protocol.	
				vanA and/or *vanB* detected	Vancomycin S	Confirm isolate identification to species level (eg, *Enterococcus faecalis*) and repeat AST. If mixed culture, test isolates individually.	If discrepancy is not resolved by suggested testing, report as vancomycin R.	1-3
				vanA and/or *vanB* not detected	Vancomycin R	Confirm isolate identification to species level (eg, *E. faecalis*) and repeat AST. If mixed culture, test isolates individually.	If discrepancy is not resolved by suggested testing, report as vancomycin R.	4

Appendix H. (Continued)

Table H2. (Continued)

Indication	Target(s)	Method	Specimen Type	Results: Genotype or Predicted Phenotype	Results: Observed Phenotype (if tested)	Suggestions for Resolution	Report as:	Comments[a]
Detection of vancomycin-resistant enterococci (Continued)	*vanA*	NAAT	Surveillance cultures	*vanA* detected	Vancomycin R	N/A	Report phenotypic result as found (if available); consider reporting presence of molecular target per institutional protocol.	1-2
				vanA not detected	Vancomycin S	N/A	Report phenotypic result as found (if available); consider reporting presence of molecular target per institutional protocol.	5
				vanA detected	Vancomycin S	Confirm isolate identification to species level (eg, *E. faecalis*) and repeat AST. If mixed culture, test isolates individually.	If the discrepancy is not resolved by suggested testing, report as vancomycin R.	1-2
				vanA not detected	Vancomycin R	Confirm isolate identification to species level (eg, *E. faecalis*) and repeat AST. If mixed culture, test isolates individually.	If the discrepancy is not resolved by suggested testing, report as vancomycin R.	4-5

Abbreviations: AST, antimicrobial susceptibility testing; N/A, not applicable; NAAT, nucleic acid amplification test; R, resistance; S, susceptible; VRE, vancomycin-resistant enterococci.

Comments

(1) *vanA* may be present in nonenterococcal species.[1]

(2) Vancomycin-variable *Enterococcus faecium* isolates were recently revealed in Canada. They carry wild-type *vanA* but initially test as vancomycin susceptible with a culture-based method. They can convert to a resistant phenotype during vancomycin treatment.[2,3]

(3) The *vanB* gene has been found in several commensal nonenterococcal bacteria, which may lead to misclassification of vancomycin-susceptible enterococci as resistant in surveillance cultures containing mixed bacterial species.[4]

Appendix H. (Continued)

Table H2. (Continued)

(4) Constitutive low-level vancomycin resistance can be detected phenotypically (2–32 µg/mL) from the presence of *vanC*, an intrinsic resistance characteristic of *Enterococcus gallinarum (vanC1*) and *Enterococcus casseliflavus* (*vanC2–C4*).[5]

(5) Targeting *vanA* only may miss regional *vanB*-carrying VRE.[6]

Footnote

a. In addition to the specific possibilities referenced in the comments, genotype and/or phenotype discrepancies could arise as a consequence of suboptimal sampling, mixed cultures, emergence of new genotypes, or mutations and/or wild-type reversions of resistance targets.

References for Table H2

1 Patel R. Enterococcal-type glycopeptide resistance genes in non-enterococcal organisms. *FEMS Microbiol Lett*. 2000;185(1):1-7.

2 Gagnon S, Lévesque S, Lefebvre B, Bourgault AM, Labbé AC, Roger M. vanA-containing *Enterococcus faecium* susceptible to vancomycin and teicoplanin because of major nucleotide deletions in Tn1546. *J Antimicrob Chemother*. 2011;66(12):2758–2762.

3 Thaker MN, Kalan L, Waglechner N, et al. Vancomycin-variable enterococci can give rise to constitutive resistance during antibiotic therapy. *Antimicrob Agents Chemother*. 2015;59(3):1405-1410.

4 Ballard SA, Grabsch EA, Johnson PD, Grayson ML. Comparison of three PCR primer sets for identification of *vanB* gene carriage in feces and correlation with carriage of vancomycin-resistant enterococci: interference by *vanB*-containing anaerobic bacilli. *Antimicrob Agents Chemother*. 2005;49(1):77-81.

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Appendix H. (Continued)

Table H3. Reporting Results From Extended-Spectrum β-Lactamase Resistance and Carbapenemase Molecular Tests for Enterobacterales

Indication	Target(s)	Method	Specimen Type	Results: Molecular Target Results	Results: Observed Phenotype (if tested)	Suggestions for Resolution	Report as:	Comments[a]
Detection of ESBL resistance in Enterobacterales (in an isolate susceptible to all carbapenems)	ESBL type CTX-M, SHV, TEM	NAAT, microarray	Colony, blood culture	Detection of any ESBL target	R to all 3rd- and 4th-generation cephalosporins tested (eg, ceftriaxone R, cefotaxime R, ceftazidime R, cefepime R)	N/A	Report phenotypic results as found (if available); consider reporting presence of molecular target per institutional protocol.	1-12
				Detection of any ESBL target	S to all 3rd- and 4th-generation cephalosporins tested (eg, ceftriaxone S, cefotaxime S, ceftazidime S, cefepime S)	Repeat molecular and phenotypic tests. If blood culture, check for mixed culture. If mixed, test isolates individually and report phenotypic results as found.	If the discrepancy is not resolved, repeat AST should be performed using a reference method, and the conflicting genotypic and phenotypic testing results should both be reported.	1-12
				Detection of *CTX-M* ESBL target	Variable resistance to 3rd- and 4th-generation cephalosporins (eg, ceftriaxone R, cefotaxime R, ceftazidime R or S, cefepime R or S)	Expected phenotype for some *CTX-M* strains. Check cefepime using a reference method if S.	Report phenotypic results as found, including reference cefepime result; consider reporting presence of molecular target per institutional protocol.	1-12
				Detection of *TEM* or *SHV* ESBL target	Variable resistance to 3rd- and 4th-generation cephalosporins (eg, ceftriaxone R or S, cefotaxime R or S, ceftazidime R or S, cefepime R or S).	Expected phenotype for some *TEM*/*SHV* strains. Check cefepime using a reference method if S.	Report phenotypic results as found, including reference cefepime result; consider reporting presence of molecular target per institutional protocol.	1-12

Appendix H. (Continued)

Table H3. (Continued)

Indication	Target(s)	Method	Specimen Type	Results		Suggestions for Resolution	Report as:	Comments[a]
				Molecular Target Results	Observed Phenotype (if tested)			
Detection of ESBL resistance in Enterobacterales (in an isolate susceptible to all carbapenems) (Continued)				No detection of ESBL targets	Resistance to 3rd-generation cephalosporins and variable resistance to 4th-generation cephalosporins (eg, ceftriaxone R, cefotaxime R, ceftazidime R, cefepime R or S)	Likely non-tested broad spectrum ß-lactamase (eg, AmpC, carbapenemase, or other ESBL); consider repeating molecular tests and checking cefepime using reference method if S.	Report phenotypic results as found, including reference cefepime result if tested.	1-12
Detection of carbapenem resistance in Enterobacterales	KPC, OXA-48-like, VIM, NDM, or IMP **Or** **Phenotypic evidence of a carbapenemase (eg, mCIM or CarbaNP positive)**	NAAT, microarray	Colony, blood culture	Detection of any tested carbapenemase target	Resistance to all carbapenems (eg, meropenem R, imipenem R, doripenem R, ertapenem R)	N/A	Report phenotypic results as found (if available); consider reporting presence of molecular target per institutional protocol.	1-4, 12-14
				Detection of any tested carbapenemase target	Susceptible to all carbapenems except ertapenem (variable) (eg, meropenem S, imipenem S, doripenem S, ertapenem R or S)	Repeat molecular and phenotypic tests. If blood culture, check for mixed culture. If mixed, test isolates individually and report phenotypic results as found; consider a phenotypic test for carbapenemase activity (such as CarbaNP or mCIM).	If the discrepancy is not resolved, repeat AST should be performed using a reference method and the conflicting genotypic and phenotypic testing results should both be reported along with a comment advising caution; current clinical and laboratory evidence is insufficient to conclude whether carbapenem monotherapy of carbapenemase-carrying strains with an MIC in the S range will be effective, or whether the molecular assays are completely accurate.	1-4, 12-15

Appendix H. (Continued)

Table H3. (Continued)

Indication	Target(s)	Method	Specimen Type	Results		Suggestions for Resolution	Report as:	Comments[a]
				Molecular Target Results	Observed Phenotype (if tested)			
Detection of carbapenem resistance in Enterobacterales (Continued)	KPC, OXA-48-like, VIM, NDM, or IMP Or Phenotypic evidence of a carbapenemase (eg, mCIM or CarbaNP positive)	NAAT, microarray	Colony, blood culture	Detection of any tested carbapenemase target or phenotypic detection of carbapenemase production	Susceptibility (S or SDD) to 3rd- and/or 4th-generation cephalosporins but intermediate or resistant to at least one carbapenem tested	Repeat molecular and phenotypic tests.	If the discrepancy is not resolved, repeat AST should be performed using a reference method, and the conflicting genotypic and phenotypic testing results should both be reported along with a comment advising caution: "Current clinical and laboratory evidence is insufficient to conclude whether cephalosporin therapy of carbapenemase-carrying strains with an MIC in the S/SDD range will be effective."	1-4, 12-14

Appendix H. (Continued)

Table H3. (Continued)

Indication	Target(s)	Method	Specimen Type	Results		Suggestions for Resolution	Report as:	Comments[a]
				Molecular Target Results	Observed Phenotype (if tested)			
Detection of carbapenem resistance in Enterobacterales (Continued)	KPC, OXA-48-like, VIM, NDM, or IMP **Or** **Phenotypic evidence of a carbapenemase (eg, mCIM or CarbaNP positive)**	NAAT, microarray	Colony, blood culture	No detection of tested carbapenemase targets	Susceptible to all carbapenems except ertapenem (eg, meropenem S, imipenem S, doripenem S, ertapenem R)	Likely ESBL/AmpC and porin alteration, especially for *Enterobacter* spp.; consider a phenotypic test for carbapenemase activity (eg, CarbaNP or mCIM); carbapenemase unlikely if negative, although rare carbapenemases (eg, GES-types, are still possible).	If carbapenemase activity is detected, repeat AST should be performed using a reference method, and the conflicting genotypic and phenotypic testing results should both be reported along with a comment advising caution; current clinical and laboratory evidence is insufficient to conclude whether carbapenem monotherapy of carbapenemase-carrying strains with an MIC in the susceptible range will be effective or whether the molecular assays are completely accurate. Otherwise report phenotypic results as found.	1-4, 12-15

Appendix H. (Continued)

Table H3. (Continued)

Indication	Target(s)	Method	Specimen Type	Results: Molecular Target Results	Results: Observed Phenotype (if tested)	Suggestions for Resolution	Report as:	Comments[a]
Detection of carbapenem resistance in Enterobacterales (Continued)	KPC, OXA-48-like, VIM, NDM, or IMP **Or** **Phenotypic evidence of a carbapenemase (eg, mCIM or CarbaNP positive)**	NAAT, microarray	Colony, blood culture	No detection of tested carbapenemase targets	Resistance to any carbapenems except ertapenem (eg, meropenem R, imipenem R, doripenem R, ertapenem R or S)	Possible other carbapenemase. If blood culture, check for mixed culture. If mixed, test isolates individually and report as found; consider repeating molecular and AST and performing a phenotypic test for carbapenemase activity (eg, CarbaNP or mCIM).	If carbapenemase activity is detected, repeat AST should be performed using a reference method, and the conflicting genotypic and phenotypic testing results should both be reported along with a comment advising caution; current clinical and laboratory evidence is insufficient to conclude whether carbapenem monotherapy of carbapenemase-carrying strains with an MIC in the S range will be effective or whether the molecular assays are completely accurate. Otherwise report phenotypic results as found.	1-4, 12-16

Abbreviations: AST, antimicrobial susceptibility testing; ESBL, extended-spectrum β-lactamase; mCIM, modified carbapenem inactivation method; MIC, minimal inhibitory concentration; N/A, not applicable; NAAT, nucleic acid amplification test; R, resistant; S, susceptible; **SDD, susceptible-dose dependent.**

<u>Comments</u>

(1) Multiple β-lactamases may be carried by individual bacterial isolates. Most carbapenemase-producing bacteria are resistant to 3rd- and 4th-generation cephalosporins, **although bacteria producing some carbapenemase enzymes (eg, OXA-48 and SME)** may not **test resistant** unless they co-produce an ESBL or AmpC **β-lactamase.**

(2) Molecular assays can detect the presence of specific β-lactamase genes but cannot exclude the presence of other β-lactamase genes or resistance mechanisms, or novel variants with changes in primer or probe annealing sites. Therefore, phenotypic resistance should always be reported.

Appendix H. (Continued)

Table H3. (Continued)

(3) Isolates with phenotypic susceptibility despite the presence of a resistance determinant may indicate the potential for resistance to emerge during therapy.

(4) These are provisional guidelines based on general principles; however, the performance characteristics of many individual research use–only assays are presently unknown.

(5) Susceptibility of TEM/SHV-carrying strains to ß-lactam combinations is variable.

(6) Susceptibility of ESBL-carrying strains to cefepime is variable.

(7) Susceptibility of ESBL-carrying strains to ß-lactam combination agents is variable.

(8) Some strains carrying CTX-M ESBLs remain susceptible to ceftazidime.

(9) Some strains carrying TEM/SHV-derived ESBLs remain susceptible to cefotaxime and ceftriaxone.

(10) Some molecular assays for AmpC may not reliably distinguish between chromosomal and plasmid-encoded genes in some bacterial species.

(11) Most strains with derepressed AmpC expression remain susceptible to cefepime.

(12) These recommendations are based on cephalosporin and carbapenem breakpoints in M100.

(13) The susceptibility to other carbapenems of ertapenem-resistant strains with ESBL or AmpC enzymes and reduced porin expression that do not contain carbapenemase genes or express carbapenemase activity may be reported as measured in phenotypic susceptibility assays.

(14) Rapid tests for carbapenemase activity (eg, CarbaNP) may not detect OXA-48-like and some other carbapenemases.

(15) Caution is advised. Current clinical evidence is insufficient to conclude whether carbapenem monotherapy of carbapenemase-carrying strains with an MIC in the susceptible range will be effective.

(16) Some isolates of Enterobacterales, in particular but not exclusively *Morganella* spp., *Proteus* spp., and *Providencia* spp., may exhibit intrinsic low-level resistance to imipenem on a non–carbapenemase-mediated basis.

Footnote

a. In addition to the specific possibilities listed in the comments, genotype and/or phenotype discrepancies could arise as a consequence of mixed cultures, emergence of new genotypes, or mutations and/or wild-type reversions of resistance targets.

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

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Appendix I. Cefiderocol Broth Preparation and Reading Broth Microdilution Minimal Inhibitory Concentration End Points

Abbreviations for Appendix I

CAMHB cation-adjusted Mueller-Hinton broth
ID-CAMHB iron-depleted cation-adjusted Mueller-Hinton broth
pH negative logarithm of hydrogen ion concentration

I1 Supplements

I1.1 Calcium and Magnesium Stock Solutions

Refer to M07[1] for cation stock solution preparation.

I1.2 Zinc Stock Solution

The steps for preparing zinc stock solution are listed below.

Step	Action	Comment
1	Dissolve 0.29 g $ZnSO_4 \cdot 7H_2O$ in 100 mL deionized water.	This solution contains **0.65** mg Zn^{++}/mL **(10 mmol Zn^{++}/mL).** **Verify that the deionized water has an iron content of ≤ 0.03 mg/L.**
2	Sterilize the solution by membrane filtration.	
3	Store the solution at 15 to 25°C **in a sterile single-use plastic container.**	**Previously used glass containers should be avoided to prevent inadvertent iron contamination.**

Appendix I. (Continued)

I2 Iron-depleted Cation-adjusted Mueller-Hinton Broth[a]

The steps for preparing iron-depleted cation-adjusted Mueller-Hinton broth (ID-CAMHB) are listed below.[2]

Step	Action	Comment
1	Prepare the CAMHB.	Follow manufacturer's instructions.
2	Autoclave the media and let cool to room temperature.	
3	Add 100 g chelating resin to 1 L autoclaved CAMHB.[2]	Removes **polyvalent metal** cations in the medium- to low-level concentrations (range, 0–0.18 mg/L).[2]
4	Stir the solution at room temperature for approximately 2 hours using a magnetic stir bar.	
5	Filter the solution using a 0.2-µm filter.	Removes the resin. **It is recommended that testing for residual iron levels of the filtrate should be conducted at this step to confirm that the iron content does not exceed 0.03 mg/L. Residual iron content can be measured with a commercial iron detection kit capable of detecting low levels of iron (0.02 mg/L). If iron levels exceed 0.03 mg/L, restart the procedure at the chelation step 3 above.**
6	Check the pH to determine whether it is 7.3 **± 0.1**.	If the pH is above **7.4**, adjust it using **1 or 6 N** HCl **(use of 6 N HCl will minimize the volume required to adjust the pH)**. If the pH is below **7.2**, use 2.5 N NaOH.
7	Add the cation to achieve final concentrations in the following ranges: • Ca^{++} 20–25 mg/L • Mg^{++} 10–12.5 mg/L • Zn^{++} 0.5–1.0 mg/L	The final concentration of **iron** in ID-CAMHB prepared using this method **should be** ≤ 0.03 mg/L. Refer to M07[1] for calculating the amount of Ca^{++}, Mg^{++}, and **the table below for calculating the amount of** Zn^{++} needed.

Appendix I. (Continued)

I2 ID-CAMBH (Continued)

Step	Action	Comment
8	Check the pH to determine whether it is 7.**3 ± 0.1**.	If the pH exceeds 7.4, adjust it using **1 or 6 N** M HCl **(use of 6 N HCl will minimize the volume required to adjust the pH).** If the pH is below 7.2, use 2.5 N NaOH.
9	Filter the final product using a 0.2-µm filter.	
10	Store the media at 4 to 8°C for up to 2 months.	

Abbreviations: CAMHB, cation-adjusted Mueller-Hinton broth; ID-CAMHB, iron-depleted cation-adjusted Mueller-Hinton broth.

Example for **adding Zn^{++} back to CAMHB** that contains below-detectable concentrations (< 0.0001 mg/L) of Zn^{++} after chelation in step 3[2]:

Step	Action	Comment
1	Calculate the amount of Zn^{++} needed using this formula: Final amount needed – amount in medium = amount to be added	For Zn^{++}, the final amount needed is 0.5-1 mg/L. 1 mg/L – 0 mg/L = 1 mg/L
2	Add **1.54** mL Zn^{++} stock per L (**1.54 mL for each** 1 mg/L).	**C = concentration, V = volume** **$C_1 \bullet V_1$ = desired $C_2 \bullet$ final V_2** **0.65 mg/mL $Zn^{++} \bullet$ V1 = 1 mg Zn^{++} /1000 mL • 1000 mL** **V_1 = 1 mg ÷ 0.65 mg/mL** **V_1= 1.54 mL of Zn^{++} stock**
3	Proceed with steps 8 and 9 above.	

Appendix I. (Continued)

I3 Determining Broth Microdilution End Points

The steps for reading and interpreting broth microdilution end points for cefiderocol when tested with ID-CAMHB are listed below.

Step	Action	Comment
1	Read the MIC as the lowest concentration of antimicrobial agent that completely inhibits organism growth in the tubes or microdilution wells as detected by the unaided eye.	See step 2 for exceptions. Viewing devices intended to facilitate reading microdilution tests and recording results may be used as long as there is no compromise in the ability to discern growth in the wells.
2	Compare the amount of growth in the wells containing the cefiderocol with the amount of growth in the growth-control well containing ID-CAMHB (no antimicrobial agent).	For a test to be considered valid, acceptable growth (definite turbidity or button) must occur in the growth-control well (see Figure I1). Trailing may occur in some organisms (eg, *Acinetobacter* spp.) and should be ignored when a tiny button or light or faint turbidity relative to the growth control may be observed. Read the MIC as the first well in which growth is significantly reduced (see Figure I2).
3	Interpret the results.	Refer to the appropriate portion of Tables 2 for breakpoints.

Abbreviations: ID-CAMHB, iron-depleted cation-adjusted Mueller-Hinton broth; MIC, minimal inhibitory concentration.

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

<u>Footnote</u>

a. **Ensure all reagents (eg, deionized water to prepare acid and base and cation solutions) have been verified as having an iron content of ≤ 0.03 mg/L.**

Appendix I. (Continued)

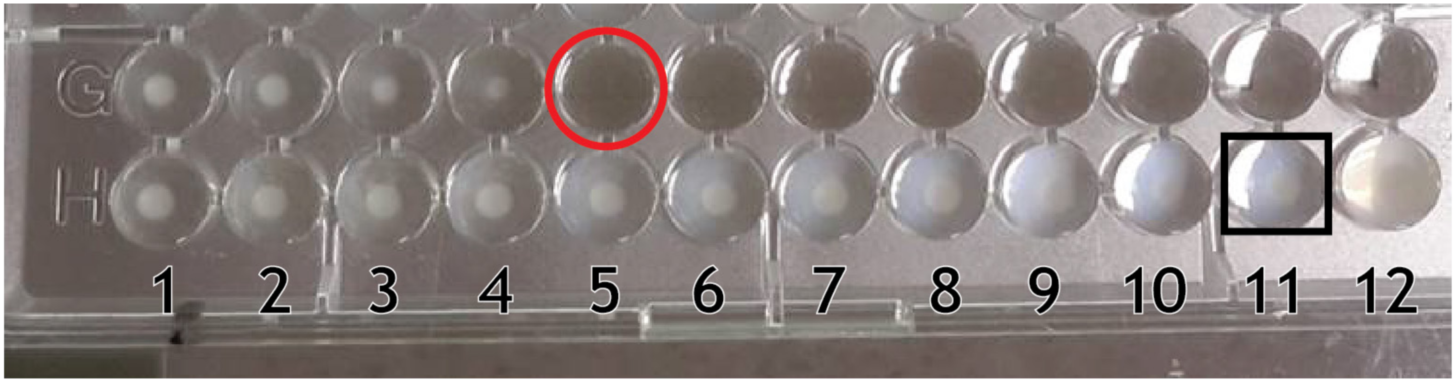

Figure I1. Cefiderocol Test With a Clear End Point. The cefiderocol concentrations in wells G1 to G12 are 0.03 to 64 µg/mL. Row G shows the cefiderocol MIC at 0.5 µg/mL in well G5 (red circle). The growth-control well is H11 (black box).

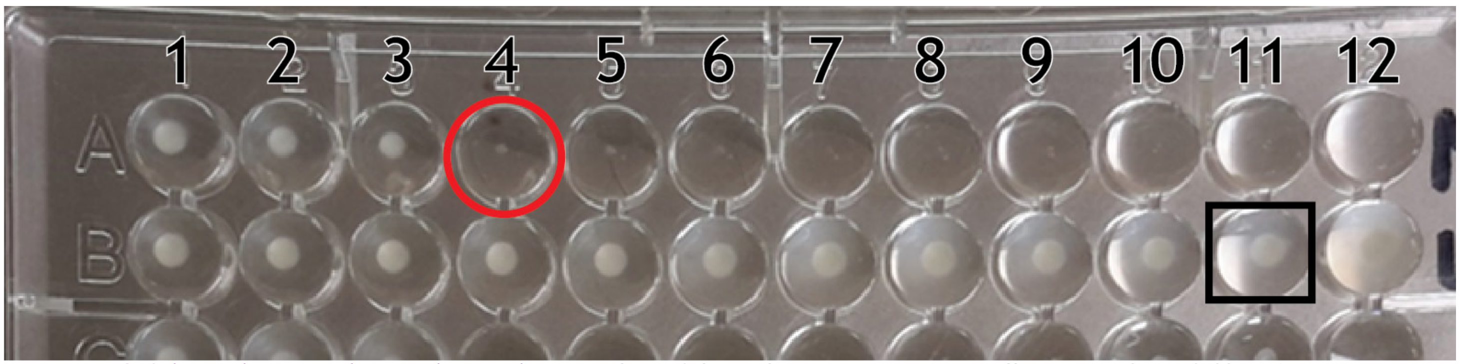

Figure I2. Cefiderocol Test With a Trailing End Point. The cefiderocol concentrations in wells A1 to A12 are 0.03 to 64 µg/mL. Row A shows the cefiderocol MIC at 0.25 µg/mL in well A4 (red circle). The growth control well is B11 (black box).

References for Appendix I

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

2 Hackel, MA, Tsuji M, Yamano Y, Echols R, Karlowsky JA, Sahm DF. Reproducibility of broth microdilution MICs for the novel siderophore cephalosporin, cefiderocol, determined using iron-depleted cation-adjusted Mueller-Hinton broth. *Diagn Microbiol Infect Dis.* 2019;94(4):321-325.

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Glossary I (Part 1). ß-Lactams: Class and Subclass Designations and Generic Names

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 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
		Aminopenicillins	Amoxicillin Ampicillin
		Carboxypenicillins	Carbenicillin Ticarcillin
		Ureidopenicillins	Azlocillin Piperacillin
	Penicillinase-stable penicillins[b]		Cloxacillin Dicloxacillin Nafcillin Oxacillin
	Aminopenicillin		Mecillinam
β-lactam combination agents			Amoxicillin-clavulanate Ampicillin-sulbactam Aztreonam-avibactam **Aztreonam-nacubactam (1:1)** Cefepime-enmetazobactam (4:1) **Cefepime-nacubactam (1:1)** Cefepime-taniborbactam Cefepime-tazobactam (1:1) Cefepime-zidebactam Ceftaroline-avibactam Ceftazidime-avibactam Ceftolozane-tazobactam Imipenem-relebactam Meropenem-nacubactam (1:1) Meropenem-vaborbactam Piperacillin-tazobactam Sulbactam-durlobactam Ticarcillin-clavulanate

Glossary I (Part 1). (Continued)

Antimicrobial Class	Antimicrobial Subclass(es)	Agent(s) Included; Generic Name(s)
Cephems (parenteral)	Cephalosporins I[c]	Cefazolin Cephalothin Cephapirin Cephradine
	Cephalosporins II[c]	Cefamandole Cefonicid Cefuroxime (parenteral)
	Cephalosporins III[c]	Cefoperazone Cefotaxime Ceftazidime Ceftizoxime Cetriaxone
	Cephalosporins IV[c]	Cefepime Cefpirome
	Cephalosporins with anti-MRSA activity	Ceftaroline Ceftobiprole
	Cephamycins	Cefmetazole Cefotetan Cefoxitin
	Oxacephem	Moxalactam
	Siderophore cephalosporin	Cefiderocol
Cephems (oral)	Cephalosporins	Cefaclor Cefadroxil Cefdinir Cefditoren Cefetamet Cefixime Cefpodoxime Cefprozil Ceftibuten Cefuroxime (oral) Cephalexin Cephradine
	Carbacephem	Loracarbef
Monobactams		Aztreonam
Penems	Carbapenems	Biapenem Doripenem Ertapenem Imipenem Meropenem Razupenem Tebipenem
	Penems	Faropenem Sulopenem

Abbreviations: MRSA, methicillin (oxacillin)-resistant *Staphylococcus aureus;* FDA, US Food and Drug Administration.

Glossary I (Part 1). (Continued)

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.

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

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Glossary I (Part 2). Non-β-Lactams: Class and Subclass Designations and Generic Names

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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
Lysins	**Lysin with antistaphylococcal activity**	Exebacase
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
	Lipoglycodepsipeptide	Ramoplanin
Lincosamides		Clindamycin Lincomycin
Lipopeptides		Daptomycin Surotomycin
	Polymyxins	Colistin Polymyxin B
Macrocyclic lactone		Fidaxomicin

Glossary I (Part 2). (Continued)

Antimicrobial Class	Antimicrobial Subclass(es)	Agent(s) Included; Generic Name(s)
Macrolides		Azithromycin Clarithromycin Dirithromycin Erythromycin
	Fluoroketolide	Solithromycin
	Ketolides	Nafithromycin Telithromycin
Nitroheterocyclics	Nitrofuran	Nitrofurantoin
	Nitroimidazoles	Metronidazole Secnidazole Tinidazole
	Thiazolides	Nitazoxanide Tizoxanide
Oxazolidinones		Linezolid Tedizolid
Peptide	Magainin	Pexiganan
Phenicols		Chloramphenicol Thiamphenicol
Pleuromutilins		Lefamulin Retapamulin
Pseudomonic acid		Mupirocin
Quinolones		Cinoxacin Garenoxacin Nalidixic acid
	Benzoquinolizine	Levonadifloxacin
	Fluoroquinolones	Besifloxacin Ciprofloxacin Clinafloxacin Delafloxacin Enoxacin Finafloxacin Fleroxacin Gatifloxacin Gemifloxacin Grepafloxacin Levofloxacin Lomefloxacin Moxifloxacin Norfloxacin Ofloxacin Ozenoxacin Pefloxacin Sparfloxacin Trovafloxacin Ulifloxacin (prulifloxacin)

Glossary I (Part 2). (Continued)

Antimicrobial Class	Antimicrobial Subclass(es)	Agent(s) Included; Generic Name(s)
Quinolonyl oxazolidinone		Cadazolid
Spiropyrimidinetrione		Zoliflodacin
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.

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

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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 some agents are no longer available for human use.

Antimicrobial Agent	Abbreviation(s)[a,b]		Route(s) of Administration[c]				Drug Class or Subclass
	CLSI Recommended	In Use	PO	IM	IV	Topical	
Amikacin	**AN**	AN, AK, Ak, AMI, AMK, **AKN**		X	X		Aminoglycoside
Amikacin-fosfomycin	**AKF**	AKF	X[d]				Aminoglycoside-fosfomycin
Amoxicillin	**AMX**	AMX, Amx, AMOX, AC, AML, **A**	X				Penicillin
Amoxicillin-clavulanate	**AMC**	AMC, Amc, A/C, AUG, Aug, XL, AML	X				β-lactam combination agent
Ampicillin	**AM**	AM, Am, AMP, **AP**	X	X	X		Penicillin
Ampicillin-sulbactam	**SAM**	SAM, A/S, AMS, AB			X		β-lactam combination agent
Azithromycin	**AZM**	AZM, Azi, AZI, AZ, **ATH**	X		X		Macrolide
Azlocillin	**AZL**	AZ, Az, AZL		X	X		Penicillin
Aztreonam	**ATM**	ATM, AZT, Azt, AT, AZM			X		Monobactam
Aztreonam-avibactam	**AZA**	AZA			X		β-lactam combination agent
Aztreonam-nacubactam	**ANC**	**ANC**			**X**		**β-lactam combination agent**
Besifloxacin	**BES**	BES				X	Fluoroquinolone
Biapenem	**BPM**	BPM			X		Carbapenem
Cadazolid	**CDZ**	CDZ	X				Quinolonyl oxazolidinone
Carbenicillin (indanyl salt) Carbenicillin	**CB**	CB, Cb, BAR, CAR, **CRB, PY**	X	X	X		Penicillin
Cefaclor	**CEC**	CEC, CCL, Cfr, FAC, CF, **CFC**	X				Cephem
Cefadroxil	**CFR**	CFR, FAD, **CDX**	X				Cephem
Cefamandole	**MA**	MA, CM, Cfm, FAM, **CMD**		X	X		Cephem

Glossary II. (Continued)

Antimicrobial Agent	Abbreviation(s)[a,b] CLSI Recommended	In Use	Route(s) of Administration[c] PO	IM	IV	Topical	Drug Class or Subclass
Cefazolin	**CZ**	CZ, CFZ, Cfz, FAZ, KZ, **CZN**		X	X		Cephem
Cefdinir	**CDR**	CDR, Cdn, DIN, CD, CFD	X				Cephem
Cefditoren	**CDN**	CDN, DIT, FD	X				Cephem
Cefepime	**FEP**	FEP, Cpe, PM, CPM		X	X		Cephem
Cefepime-enmetazobactam	**FPE**	FPE			X		β-lactam combination agent
Cefepime-nacubactam	**CNC**	**CNC**			**X**		**β-lactam combination agent**
Cefepime-taniborbactam	**FTB**	FTB			X		β-lactam combination agent
Cefepime-tazobactam	**FPT**	FPT			X		β-lactam combination agent
Cefepime-zidebactam	**FPZ**	FPZ			X		β-lactam combination agent
Cefetamet	**CAT**	CAT, FET	X				Cephem
Cefiderocol	**FDC**	FDC			X		Siderophore β-lactam
Cefixime	**CFM**	CFM, FIX, Cfe, IX	X				Cephem
Cefmetazole	**CMZ**	CMZ, CMZS, CMT, Cmz		X	X		Cephem
Cefonicid	**CID**	CID, Cfc, FON, CPO		X	X		Cephem
Cefoperazone	**CFP**	CFP, Cfp, CPZ, PER, FOP, CP		X	X		Cephem
Cefotaxime	**CTX**	CTX, TAX, Cft, FOT, CT		X	X		Cephem
Cefotetan	**CTT**	CTT, CTN, Ctn, CTE, TANS, CN		X	X		Cephem
Cefoxitin	**FOX**	FOX, CX, Cfx, FX		X	X		Cephem
Cefpirome	**CPO**	CPO, CPR, CR		X	X		Cephem
Cefpodoxime	**CPD**	CPD, Cpd, POD, PX	X				Cephem
Cefprozil	**CPR**	CPR, CPZ, FP	X				Cephem
Ceftaroline	**CPT**	CPT, Cpt, **CTR**			X		Cephem
Ceftaroline-avibactam	**CPA**	CPA			X		β-lactam combination agent
Ceftazidime	**CAZ**	CAZ, Caz, TAZ, TZ		X	X		Cephem
Ceftazidime-avibactam	**CZA**	CZA			X		β-lactam combination agent

Glossary II. (Continued)

Antimicrobial Agent	Abbreviation(s)[a,b]		Route(s) of Administration[c]				Drug Class or Subclass
	CLSI Recommended	In Use	PO	IM	IV	Topical	
Ceftibuten	**CTB**	CTB, TIB, CB, **CFB, CFT**	X				Cephem
Ceftizoxime	**ZOX**	ZOX, CZX, CZ, Cz, CTZ, TIZ		X	X		Cephem
Ceftobiprole	**BPR**	BPR			X		Cephem
Ceftolozane-tazobactam	**CT**	CT, C/T, CXT, **CLT**			X		β-lactam combination agent
Ceftriaxone	**CRO**	CRO, CTR, FRX, Cax, AXO, TX		X	X		Cephem
Cefuroxime (oral) Cefuroxime (parenteral)	**CXM**	CXM, CFX, ROX, Crm, FUR, XM	X	 X	 X		Cephem
Cephalexin	**CN**	CN, LEX, CFL, CL, **CFX**	X				Cephem
Cephalothin	**CF**	CF, Cf, CR, CL, CEP, CE, KF, **CEF**			X		Cephem
Cephapirin	**CP**	CP, HAP		X	X		Cephem
Cephradine	**RAD**	RAD, CH, CED, CE	X				Cephem
Chloramphenicol	**C**	C, CHL, CL	X		X		Phenicol
Cinoxacin	**CIN**	CIN, Cn	X				Quinolone
Ciprofloxacin	**CIP**	CIP, Cp, CI	X		X		Fluoroquinolone
Clarithromycin	**CLR**	CLR, CLM, CLA, Cla, CH	X				Macrolide
Clinafloxacin	**CLX**	CFN, CLX, LF, **CFL**	X		X		Fluoroquinolone
Clindamycin	**CM**	CC, CM, CD, Cd, CLI, DA	X	X	X		Lincosamide
Cloxacillin	**CLO**	**CX, Clx, CLO, OB, OX**	**X**	**X**	**X**		**Penicillin**
Colistin	**CL**	CL, CS, CT, CI, CO, COL			X		Lipopeptide
Dalbavancin	**DAL**	DAL			X		Lipoglycopeptide
Daptomycin	**DAP**	DAP, Dap, DPC			X		Lipopeptide
Delafloxacin	**DLX**	DLX, **DFX**	X		X		Fluoroquinolone
Dicloxacillin	**DX**	DX, DIC	X				Penicillin
Dirithromycin	**DTM**	DTM, DT, DIR	X				Macrolide
Doripenem	**DOR**	DOR, Dor			X		Carbapenem
Doxycycline	**DO**	DO, DOX, DC, DOXY, D, DX, Dox, **DXT**	X		X		Tetracycline

Glossary II. (Continued)

Antimicrobial Agent	Abbreviation(s)[a,b] CLSI Recommended	In Use	Route(s) of Administration[c] PO	IM	IV	Topical	Drug Class or Subclass
Enoxacin	**ENX**	**ENX, Enx, ENO, ENOX, ENO(F)**	**X**				**Fluoroquinolone**
Ertapenem	**ETP**	ETP, Etp		X	X		Carbapenem
Eravacycline	**ERV**	ERV	X		X		Fluorocycline
Erythromycin	**E**	E, ERY, EM	X		X		Macrolide
Exebacase	**EXE**	EXE			X		Antistaphylococcal lysin
Faropenem	**FPM**	FAR, FARO, FPM, Faro	X				Penem
Fidaxomicin	**FDX**	FDX	X				Macrocyclic
Finafloxacin	**FIN**	FIN	X		X	X	Fluoroquinolone
Fleroxacin	**FLE**	FLE, Fle	X		X		Fluoroquinolone
Fosfomycin	**FOS**	FOS, FF, FO, FM, Fos	X				Fosfomycin
Fusidic acid	**FA**	FA, FC, FUS, FD, **FU, FAD**	X		X	X	Steroidal
Garenoxacin	**GRN**	GRN, Grn	X		X		Quinolone
Gatifloxacin	**GAT**	GAT, Gat, **GA, GFLX**	X		X		Fluoroquinolone
Gemifloxacin	**GEM**	GEM, Gem	X				Fluoroquinolone
Gentamicin Gentamicin synergy	**GM**	GM, Gm, CN, GEN GM500, HLG, Gms, GHLR, GMS		X	X		Aminoglycoside
Gepotidacin	**GEP**	GEP	X		X		Triazaacenaphthylene
Grepafloxacin	**GRX**	GRX, Grx, GRE, GP	X				Fluoroquinolone
Iclaprim	**ICL**	ICL, IP			X		Folate pathway antagonist
Imipenem	**IPM**	IPM, IMI, Imp, IP			X		Carbapenem
Imipenem-relebactam	**IMR**	IMR, **IPR, I/R**			X		β-lactam combination agents
Kanamycin	**K**	K, KAN, HLK, KM		X	X		Aminoglycoside
Lefamulin	**LMU**	LMU	X		X		Pleuromutilin
Levofloxacin	**LVX**	LVX, Lvx, LEV, LEVO, LE	X		X		Fluoroquinolone
Levonadifloxacin	**LND**	LND			X		Benzoquinolizine
Lincomycin	**LIN**	**L, Lin, LIN, MY**		**X**	**X**		**Lincosamide**
Linezolid	**LZD**	LNZ, LZ, LZD, Lzd	X		X		Oxazolidinone
Lomefloxacin	**LOM**	LOM, Lmf, **LFLX, LOMX**	X				Fluoroquinolone
Loracarbef	**LOR**	LOR, Lor	X				Cephem

Glossary II. (Continued)

Antimicrobial Agent	Abbreviation(s)[a,b]		Route(s) of Administration[c]				Drug Class or Subclass
	CLSI Recommended	In Use	PO	IM	IV	Topical	
Mecillinam	**MEC**	MEC, Mec, **MM, MEL**	X				Penicillin
Meropenem	**MEM**	MEM, Mer, MERO, MRP, MP			X		Carbapenem
Meropenem-nacubactam	**MNC**	MNC			X		β-lactam combination agent
Meropenem-vaborbactam	**MEV**	MEV			X		β-lactam combination agent
Methicillin	**ME**	**ME, MET, DP**		**X**	**X**		**Penicillin**
Metronidazole	**MET**	MET, MTZ, MZ, MRD, MTR	X		X		Nitroimidazole
Minocycline	**MI**	MI, MIN, Min, MN, MNO, MC, MH	X		X		Tetracycline
Moxalactam	**MOX**	MOX, Mox		X	X		Cephem
Moxifloxacin	**MXF**	MXF, Mxf, MX	X		X		Fluoroquinolone
Mupirocin	**MUP**	MUP, MOP, MU, Mup, **PUM**				X	Pseudomonic acid
Nafcillin	**NF**	NF, NAF, Naf		X	X		Penicillin
Nafithromycin	**ZMK**	ZMK, **ZWK**	X				Ketolide
Nalidixic acid	**NA**	NA, NAL	X				Quinolone
Netilmicin	**NET**	NET, Nt, NC		X	X		Aminoglycoside
Nitazoxanide	**NIT**	NIT	X				Thiazolide
Nitrofurantoin	**FM**	FM, F/M, FD, Fd, FT, NIT, NI, F	X				Nitrofuran
Norfloxacin	**NX**	NX, **NOV, NV, NO**	X				Fluoroquinolone
Novobiocin	**NB**	**NB**				**X**	**Aminocoumarin**
Ofloxacin	**OFL**	OFL, OFX, Ofl, OF	X	X	X		Fluoroquinolone
Omadacycline	**OMC**	OMC	X		X		Tetracycline
Oritavancin	**ORI**	ORI			X		Lipoglycopeptide
Oxacillin	**OX**	OX, Ox, OXS, OXA	X	X	X		Penicillin
Ozenoxacin	**OZN**	OZN				X	Fluoroquinolone
Pefloxacin	**PEF**	PEF, PF, Pef, PE					Fluoroquinolone
Penicillin	**P**	P, PEN, PV, PG	X	X	X		Penicillin
Pexiganan	**PEX**	PEX, P/N				X	Peptide

Glossary II. (Continued)

Antimicrobial Agent	Abbreviation(s)[a,b]		Route(s) of Administration[c]				Drug Class or Subclass
	CLSI Recommended	In Use	PO	IM	IV	Topical	
Piperacillin	**PIP**	PIP, PI, PP, Pi, PRL		X	X		Penicillin
Piperacillin-tazobactam	**TZP**	TZP, PTZ, P/T, PTc			X		β-lactam combination agent
Plazomicin	**PLZ**	PLZ			X		Aminoglycoside
Polymyxin B	**PB**	PB, POL, PO			X		Lipopeptide
Quinupristin-dalfopristin	**SYN**	SYN, Syn, QDA, RP, **QDF**			X		Streptogramin
Ramoplanin	**RAM**	RAM	X				Lipoglycodepsipeptide
Razupenem	**RZM**	RZ, RZM			X		Carbapenem
Rifampin	**RA**	RA, RIF, Rif, RI, RD, **RP, RFP**	X		X		Ansamycin
Rifamycin	**RIF**	**RF, RIF**	**X**		**X**		**Ansamycin**
Rifaximin	**RFP**	RFP	X				Ansamycin
Secnidazole	**SEC**	SEC	X				Nitroimidazole
Solithromycin	**SOL**	SOL	X		X	X	Fluoroketolide
Sparfloxacin	**SPX**	SPX, Sfx, **SPX,** SO, **SPFX**	X				Fluoroquinolone
Spectinomycin	**SPT**	SPT, SPE, SC, SP, **SH, SPC**		X	X		Aminocyclitol
Streptomycin Streptomycin synergy	**STS**	STS, S, STR, StS, SM, ST2000, HLS, **SHLR**		X	X		Aminoglycoside
Sulbactam-durlobactam	**SUD**	SUD**, SUL**		**X**			ß-lactam combination agent
Sulfonamides	**SSS**	G, SSS, S3	X		X		Folate pathway antagonist (some PO only)
Sulopenem	**SLP**	SLP, **SPM**	X		X		Penem
Surotomycin	**SUR**	SUR	X				Lipopeptide
Tebipenem	**TBP**	TBP	X				Carbapenem
Tedizolid	**TZD**	TZD	X		X		Oxazolidinone
Teicoplanin	**TEC**	TEC, TPN, Tei, TEI, TP, TPL		X	X		Lipoglycopeptide
Telavancin	**TLV**	TLV, **TLA**			X		Lipoglycopeptide
Telithromycin	**TEL**	TEL	X				Ketolide
Tetracycline	**TE**	TE, Te, TET, TC	X		X		Tetracycline

Glossary II. (Continued)

Antimicrobial Agent	Abbreviation(s)[a,b]		Route(s) of Administration[c]				Drug Class or Subclass
	CLSI Recommended	In Use	PO	IM	IV	Topical	
Thiamphenicol	**TP**	**TP**	**X**	**X**	**X**		**Phenicol**
Ticarcillin	**TIC**	TIC, TC, TI, Ti		X	X		Penicillin
Ticarcillin-clavulanate	**TIM**	TIM, Tim, T/C, TCC, TLc, **TTC**			X		β-lactam combination agent
Tigecycline	**TGC**	TGC, Tgc			X		Glycylcycline
Tinidazole	**TNZ**	TNZ	X				Nitroimidazoles
Tinoxanide	**TIN**	TIN	X				Thiazolide
Tobramycin	**TM**	TM, NN, TO, To, TOB, **TN**		X	X		Aminoglycoside
Trimethoprim	**TMP**	TMP, T, TR, W,**TM**	X				Folate pathway antagonist
Trimethoprim-sulfamethoxazole	**SXT**	SXT, SxT, T/S, TS, COT	X		X		Folate pathway antagonist
Trospectomycin	**TBR**	TBR		X	X		Aminocyclitol
Trovafloxacin	**TRO**	TVA, Tva, TRV, TV, TRO	X		X		Fluoroquinolone
Ulifloxacin (prulifloxacin)	**PRU**	PRU, **ULI**	X				Fluoroquinolone
Vancomycin	**VA**	VA, Va, VAN, **VCM**	X		X		Glycopeptide
Zoliflodacin	**ZFD**	ZFD	X				Spiropyriminetrione

Abbreviations: **AST, antimicrobial susceptibility testing; combo, in combination**; FDA, US Food and Drug Administration; IM, intramuscular; IV, intravenous; PO, oral.

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. Abbreviations used by AST device manufacturers may differ from those recommended by CLSI.

c. As available in the United States.

d. Amikacin-fosfomycin is aerosolized and inhaled.

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

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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 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
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 and Leadership • Customer Focus • Facilities and Safety Management • Personnel Management	• Supplier and Inventory Management • Equipment Management • Process Management • Documents and Records Management	• Information Management • Nonconforming Event Management • Assessments • Continual Improvement

The QSEs covered by M100 and its related CLSI documents are available on the CLSI website: https://clsi.org/qse

Related CLSI Reference Materials[a]

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.

M02QG **M02 Disk Diffusion Reading Guide. 1st ed., 2018.** The Disk Diffusion Reading Guide provides photographic examples of the proper method for reading disk diffusion susceptibility testing results.

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. 9th ed., 2018.** This standard provides reference methods for determining 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.

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

NOTES

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