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

Testing Conditions

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

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

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

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

N. gonorrhoeae ATCC®* 49226

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

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

General Comments

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

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

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

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

Table 2F. *Neisseria gonorrhoeae* (Continued)

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

Table 2F. *Neisseria gonorrhoeae* (Continued)

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

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

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) Isolates with disk zone diameters ≤19 mm usually indicate plasmid-mediated tetracycline resistance.** 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.

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. Their *in vitro* activity is best determined using an MIC method.

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

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

Table 2G. *Streptococcus pneumoniae* (Continued)

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

Table 2G. *Streptococcus pneumoniae* (Continued)

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

Table 2G. *Streptococcus pneumoniae* (Continued)

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

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

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

Testing Conditions

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

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

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

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

S. pneumoniae ATCC®* 49619

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

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

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

General Comments

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

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

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

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

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
PENICILLINS **(6)** An organism that is susceptible to penicillin can be considered susceptible to antimicrobial agents listed here when used for approved indications and does not need to be tested against those agents. For groups A, B, C, and G β-hemolytic streptococci, penicillin is a surrogate for ampicillin, amoxicillin, amoxicillin-clavulanate, ampicillin-sulbactam, cefazolin, cefepime, ceftaroline, cephradine, cephalothin, cefotaxime, ceftriaxone, ceftizoxime, imipenem, ertapenem, and meropenem. For group A β-hemolytic streptococci, penicillin is also a surrogate for cefaclor, cefdinir, cefprozil, ceftibuten, cefuroxime, and cefpodoxime.									
A	Penicillin or	10 units	≥24	–	–	≤0.12	–	–	See general comment (4).
A	ampicillin	10 µg	≥24	–	–	≤0.25	–	–	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.) See comment (6).									
B	Cefepime or	30 µg	≥24	–	–	≤0.5	–	–	
B	cefotaxime or	30 µg	≥24	–	–	≤0.5	–	–	
B	ceftriaxone	30 µg	≥24	–	–	≤0.5	–	–	
C	Ceftaroline	30 µg	≥26	–	–	≤0.5	–	–	**(7)** Breakpoints are based on a dosage regimen of 600 mg 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 **3H**.
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 **3H**, 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)** Report against *S. pyogenes*.
OXAZOLIDINONES									
C	Linezolid	30 µg	≥21	–	–	≤2	–	–	
C	Tedizolid	–	–	–	–	≤0.5	–	–	**(16)** For reporting against *S. pyogenes* and *S. agalactiae* only.

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

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

Testing Conditions

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

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

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

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

S. pneumoniae ATCC®* 49619

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

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

General Comments

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

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

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

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

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

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

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

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

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

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

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

Testing Conditions

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

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

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

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

Streptococcus pneumoniae ATCC®* 49619:

Disk diffusion: incubate in 5% CO_2.

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

E. coli ATCC® 25922

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

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

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

General Comments

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

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

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

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

Table 2I. *Neisseria meningitidis* (Continued)

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

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

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

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

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.

ESBL

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 twofold concentration decrease in an MIC for either antimicrobial agent tested in combination with clavulanate vs the MIC of the agent when tested alone = ESBL (eg, ceftazidime MIC = 8 μg/mL; ceftazidime-clavulanate MIC = 1 μg/mL).
Reporting			For all confirmed ESBL-producing strains: If laboratories do not use current cephalosporin and aztreonam breakpoints, the test interpretation should be reported as resistant for all penicillins, cephalosporins, and aztreonam. If laboratories use current cephalosporin and aztreonam breakpoints, 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). *E. coli* ATCC® 25922 (see acceptable QC ranges in Table 4A-1) *K. pneumoniae* ATCC® 700603: Cefpodoxime zone 9–16 mm Ceftazidime zone 10–18 mm Aztreonam zone **10–16** mm Cefotaxime zone 17–25 mm Ceftriaxone zone 16–24 mm	When testing antimicrobial agents used for ESBL detection, *K. pneumoniae* ATCC® 700603 is provided as a supplemental QC strain (eg, for training, competence assessment, or test evaluation). Either strain, *K. pneumoniae* ATCC® 700603 or *E. coli* ATCC® 25922, may then be used for routine QC (eg, weekly or daily). *E. coli* ATCC® 25922 = no growth (see acceptable QC ranges listed in Table 5A-1) *K. pneumoniae* ATCC® 700603 = Growth: Cefpodoxime MIC ≥ 8 μg/mL Ceftazidime MIC ≥ 2 μg/mL Aztreonam MIC ≥ 2 μg/mL Cefotaxime MIC ≥ 2 μg/mL Ceftriaxone MIC ≥ 2 μg/mL	When performing the ESBL test, *K. pneumoniae* ATCC® 700603 and *E. coli* ATCC® 25922 should be used for routine QC (eg, weekly or daily). **Acceptable QC:** *E. coli* ATCC® 25922: ≤ 2-mm increase in zone diameter for antimicrobial agent tested in combination with clavulanate vs the zone diameter when tested alone. *K. pneumoniae* ATCC® 700603: ≥ 5-mm increase in zone diameter of ceftazidime-clavulanate vs ceftazidime alone; ≥ 3-mm increase in zone diameter of cefotaxime-clavulanate vs cefotaxime alone.	When performing the ESBL test, *K. pneumoniae* ATCC® 700603 and *E. coli* ATCC® 25922 should be tested routinely (eg, weekly or daily). **Acceptable QC:** *E. coli* ATCC® 25922: < 3 twofold concentration decrease in MIC for antimicrobial agent tested in combination with clavulanate vs the MIC of the agent when tested alone. *K. pneumoniae* ATCC® 700603: ≥ 3 twofold concentration decrease in MIC for an antimicrobial agent tested in combination with clavulanate vs the MIC of the agent when tested alone.

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

Tests for Carbapenemases in *Enterobacteriaceae and Pseudomonas aeruginosa*

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

Table 3E-1. 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 and *Pseudomonas aeruginosa*
Medium	MHA
Antimicrobial concentration	Standard disk contents for the antimicrobials are detailed in Table 3E-2 (Enterobacterales) and Table 3E-3 (*P. aeruginosa*)
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	8-10 hours or 16–18 hours **(refer to Tables 3E-2 and 3E-3 for antimicrobial agent-specific incubation lengths)**
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 3E-2 or Table 3E-3 if the gram-negative bacillus tested is confirmed to be an Enterobacterales or *P. aeruginosa*, respectively. If species is identified as another organism, do not interpret or report results.

Table 3E-1. (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	• Perform QC according to the standard disk diffusion QC procedures per M02[1] (eg, daily or weekly). • See Tables 4A-1 and 4A-2 for acceptable QC ranges. • *E. coli* ATCC®[a] 25922, *P. aeruginosa* ATCC® 27853 • Refer to Table 4A-2 to select strains for routine QC of β-lactam combination agents.

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

Table 3E-2. Enterobacterales (Continued)

Antimicrobial Agent	Disk Content	Read Times, hours	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Comments
			S	SDD	I	R	
PENICILLINS							
Ampicillin	10 µg	8-10	**≥16**	-	**12-15**	**≤ 11**	**(4)** Results of ampicillin testing can be used to predict results for amoxicillin.
		16-18	≥ 17	-	14-16	≤ 13	**(5)** Breakpoints are based on an ampicillin dosage regimen of 2 g parenterally administered every 4-6 h or an amoxicillin dosage regimen of 1-2 g parenterally administered every 6 h.
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)							
Ceftriaxone	30 µg	8-10	≥ 23	-	20-22	≤ 19	**(6)** Breakpoints are based on a dosage regimen of 1 g administered every 24 h.
		16-18	≥ 23	-	20-22	≤ 19	
Ceftazidime	30 µg	8-10	≥ 21	-	18-20	≤ 17	**(7)** Breakpoints are based on a dosage regimen of 1 g administered every 8 h.
		16-18	≥ 21	-	18-20	≤ 17	
MONOBACTAMS							
Aztreonam	30 µg	8-10	≥ 21	-	18-20	≤ 17	**(8)** Breakpoints are based on a dosage regimen of 1 g administered every 8 h.
		16-18	≥ 21	-	18-20	≤ 17	
CARBAPENEMS							
Meropenem	**10 µg**	**8-10**	**≥22**	**-**	**20-21**	**≤ 19**	
		16-18	**≥22**	**-**	**19-21**	**≤ 18**	
AMINOGLYCOSIDES							
Tobramycin	10 µg	8-10	≥ 15	-	13-14	≤ 12	
		16-18	≥ 15	-	13-14	≤ 12	
FLUOROQUINOLONES for Enterobacterales except *Salmonella* spp.							
Ciprofloxacin	**5 µg**	**8-10**	**≥ 21**	**-**	**18-20**	**≤ 17**	
		16-18	**≥ 21**	**-**	**18-20**	**≤ 17**	
FOLATE PATHWAY ANTAGONISTS							
Trimethoprim-sulfamethoxazole	1.25/ 23.75 µg	8-10	-	-	-	-	
		16-18	≥16	-	11-15	≤ 10	

Abbreviations: I, intermediate; MIC, minimal inhibitory concentration; R, resistant; S, susceptible; SDD, susceptible-dose dependent.

Table 3E-3. Zone Diameter Disk Diffusion Breakpoints for *Pseudomonas aeruginosa* Direct From Blood Culture

General Comments

(1) Organism identification must be known before interpreting and reporting results.

(2) The dosage regimens shown in the Comments column below are necessary to achieve plasma drug exposure (in adults with normal renal and hepatic function) on which breakpoints were derived. When new breakpoints are implemented, it is strongly recommended that laboratories share this information with the antimicrobial stewardship team **and other relevant institutional stakeholders.**

(3) For additional testing and reporting recommendations, refer to Table 2B-1.

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

Antimicrobial Agent	Disk Content	Read Times, hours	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Comments
			S	SDD	I	R	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)							
Ceftazidime	30 µg	8-10	-	-	-	-	**(4)** Breakpoints are based on a dosage regimen of 1 g administered every 6 h or 2 g administered every 8 h.
		16-18	≥ 18	-	15-17	≤ 14	
CARBAPENEMS							
Meropenem	10 µg	**8-10**	**≥ 19**	-	**16-18**	**≤ 15**	**(5)** Breakpoints are based on a dosage regimen of 1 g administered every 8 h.
		16-18	≥ 19	-	16-18	≤ 15	
AMINOGLYCOSIDES							
Tobramycin	10 µg	8-10	≥ 15	-	13-14	≤ 12	
		16-18	≥ 15	-	13-14	≤ 12	
FLUOROQUINOLONES							
Ciprofloxacin	5 µg	8-10	≥ 23	-	18-22	≤ 17	**(6)** Breakpoints are based on a dosage regimen of 400 mg administered parenterally every 8 h.
		16-18	≥ 25	-	19-24	≤ 18	

Abbreviations: I, intermediate; R, resistant; S, susceptible; SDD, susceptible-dose dependent.

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

Test	β-Lactamase Production	
Test method	**Disk Diffusion (penicillin zone-edge test)**	**Nitrocefin-based Test**
Organism group	*S. aureus* with penicillin MICs ≤ 0.12 µg/mL or zones ≥ 29 mm[a]	***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") = β-lactamase positive (see Figure 1 below this table) Fuzzy zone edge ("beach") = β-lactamase negative (see Figure 2 below this table)	Nitrocefin-based test: conversion from yellow to red/pink = β-lactamase positive.
Additional testing and reporting	β-lactamase-positive staphylococci are resistant to penicillin, amino-, carboxy-, and ureidopenicillins.	Nitrocefin-based tests can be used for *S. aureus,* but negative results should be confirmed with the penicillin zone-edge test before reporting penicillin as susceptible. β-lactamase-positive staphylococci are resistant to penicillin, amino-, carboxy-, and ureidopenicillins.
QC recommendations – routine[c]	*S. aureus* ATCC®[d] 25923 for routine QC of penicillin disk to include examination of zone-edge test (fuzzy edge = "beach")	
QC recommendations – lot/shipment[e]		*S. aureus* ATCC® 29213 – positive *S. aureus* ATCC® 25923 – negative (or see local regulations and manufacturers' recommendations)
QC recommendations – supplemental[f]	*S. aureus* ATCC® 29213 – positive penicillin zone-edge test (sharp edge = "cliff")	

Abbreviations: ATCC®, American Type Culture Collection; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; N/A, not applicable; QC, quality control.

Footnotes

The penicillin disk diffusion zone-edge test was shown to be more sensitive than nitrocefin-based tests for detection of ß-lactamase production in *S. aureus.*The penicillin zone-edge test is recommended if only one test is used for ß-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 ß-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)

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

Table 3E. (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.

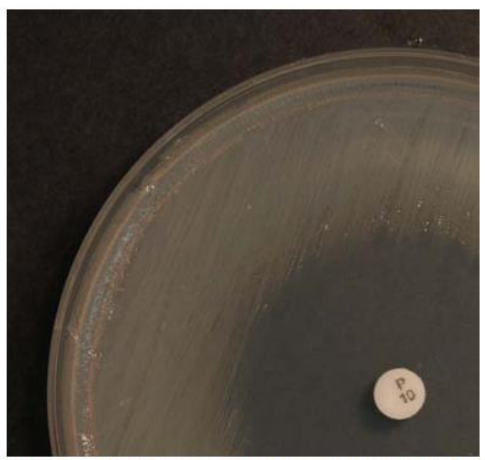


Figure 2. Negative Penicillin Disk Zone-Edge Test for β-Lactamase Detection. The zone edge is fuzzy or like a “beach,” indicating no β-lactamase production.

Table 3H. 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 3H. (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 β-hemolytic *Streptococcus* spp.	**All *Staphylococcus* spp.**[c]	*S. pneumoniae* and β-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)	

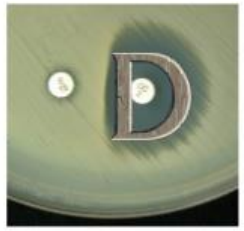
Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; **ICR, inducible clindamycin resistance;** LHB, lysed horse blood; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; TSA, tryptic soy agar.

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

The “D” Test

- Is Staph aureus really susceptible to Clindamycin
- Why?? During therapy, S aureus isolates resistant to Erythromycin possess enzymes capable of inducing Clindamycin resistance
- Kirby Bauer zone around Clindamycin will be blunted to form a “D” if Clindamycin can be induced by Erythromycin to be resistant – so called **INDUCIBLE RESISTANCE**.
- Clindamycin should be reported as resistant by clindamycin induction and not used for therapy.

D test **positive** Inducible resistance



D test **negative** Clindamycin can be used for therapy