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 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.

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	Ciprofloxacin	5 µg	≥21	16-20^	≤15	≤1	-	2^	≥4	
U	Levofloxacin	5 µg	≥17	14-16^	≤13	≤2		4^	≥8	
O	Gatifloxacin	5 µg	≥18	15-17^	≤14	≤2	-	4^	≥8	
O	Norfloxacin	10 µg	≥17	13-16	≤12	≤4	-	8	≥16	**(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.