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) Refer to Table 1I for antimicrobial agents that should be considered for testing and reporting by microbiology laboratories.

(2) 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.

(3) 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).

(4) **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.

(5) 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).

(6) An intermediate (I) with a ^ in Tables 2 indicates agents that have the potential to concentrate in the urine. The I^ is for informational use only. The decision to report I^ is best made by each laboratory based on institution-specific guidelines and in consultation with appropriate medical personnel.

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

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

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									
Penicillin	10 units	≥ 15	-	≤ 14	≤ 8	-	-	≥ 16	**(7)** 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-ß-lactamase-producing enterococci. Ampicillin susceptibility can be used to predict imipenem susceptibility, providing the species is confirmed to be *E. faecalis*. **(8)** 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, testing of penicillin is required. **(9)** ***Rx:*** Combination therapy with high-dosage parenteral ampicillin, amoxicillin, 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. **(10)** 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. **(11)** Breakpoints when oral ampicillin is used for therapy of uncomplicated UTIs only are based on an ampicillin dosage regimen of 500 mg orally administered every 6 h or amoxicillin dosage regimen of 250 mg orally administered every 8 h or 500 mg every 12 h.
Ampicillin	10 µg	≥ 17	-	≤ 16	≤ 8	-	-	≥ 16	

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

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 (Continued)									
Penicillin	10 units	≥15	-	≤14	≤8	-	-	≥16	**(12)** 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).
Ampicillin	10 µg	≥17	-	≤16	≤8	-	-	≥16	
GLYCOPEPTIDES									
Vancomycin	30 µg	≥17	15-16	≤14	≤4	-	8-16	≥32	**(13)** 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 (5) and comment (9).

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

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	
LIPOGLYCOPEPTIDES									
Dalbavancin	-	-	-	-	≤ 0.25	-	-	-	**(14) Report only on** vancomycin-susceptible *E. faecalis*. **(15)** Breakpoints are based on a dosage regimen of 1500 mg (single dose) or 1000 mg (two doses) IV administered over 30 minutes followed one week later by 500 mg IV administered over 30 minutes.
Oritavancin	-	-	-	-	≤ 0.12	-	-	-	**(16)** Breakpoints are based on a dosage regimen of 1200 mg administered IV once. See comment (14).
Telavancin	-	-	-	-	≤ 0.25	-	-	-	**(17)** Breakpoints are based on a dosage regimen of 10 mg/kg administered every 24 h. See comment (14).
Teicoplanin **(Inv.)**	30 µg	≥ 14	11-13	≤ 10	≤ 8	-	16	≥ 32	
LIPOPEPTIDES									
Daptomycin *E. faecium* only	-	-	-	-	-	≤ 4	-	≥ 8	**(18) Not routinely** reported **on organisms isolated** from the respiratory tract. **(19)** 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.
Daptomycin *Enterococcus* spp. other than *E. faecium*	-	-	-	-	≤ 2	-	4	≥ 8	**(20)** The breakpoint for susceptible is based on a dosage regimen of 6 mg/kg administered every 24 h. See comment (18).

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

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	
MACROLIDES									
Erythromycin*	15 µg	≥23	14-22	≤13	≤0.5	-	1-4	≥8	**(21)** Not routinely reported on **organisms isolated** from the urinary tract.
TETRACYCLINES									
(22) 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.									
Tetracycline **(U)**[b]	30 µg	≥19	15-18	≤14	≤4	-	8	≥16	
Doxycycline*	30 µg	≥16	13-15	≤12	≤4	-	8	≥16	
Minocycline*	30 µg	≥19	15-18	≤14	≤4	-	8	≥16	
FLUOROQUINOLONES									
Ciprofloxacin **(U)**[b]	5 µg	≥21	16-20^	≤15	≤1	-	2^	≥4	
Levofloxacin **(U)**[b]	5 µg	≥17	14-16^	≤13	≤2		4^	≥8	
Gatifloxacin*	5 µg	≥18	15-17^	≤14	≤2	-	4^	≥8	
Norfloxacin* **(U)**[b]	10 µg	≥17	13-16	≤12	≤4	-	8	≥16	
NITROFURANS									
Nitrofurantoin **(U)**[b]	300 µg	≥17	15-16	≤14	≤32	-	64	≥128	
ANSAMYCINS									
Rifampin*	5 µg	≥20	17-19	≤16	≤1	-	2	≥4	**(23)** ***Rx:*** Rifampin should not be used alone for antimicrobial therapy.
FOSFOMYCINS									
Fosfomycin **(U)**[b]	200 µg	≥16	13-15	≤12	≤64	-	128	≥256	**(24) Report only on *E. faecalis*.** **(25)** 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. **(26)** The 200-µg fosfomycin disk contains 50 µg glucose-6-phosphate.

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

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	
PHENICOLS									
Chloramphenicol*****	30 µg	≥18	13-17	≤12	≤8	-	16	≥32	See comment (21).
STREPTOGRAMINS									
Quinupristin-dalfopristin*****	15 µg	≥19	16-18	≤15	≤1	-	2	≥4	**(27)** **Report only on** vancomycin-resistant *E. faecium*.
OXAZOLIDINONES									
(28) *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.									
Linezolid	30 µg	≥23	21-22	≤20	≤2	-	4	≥8	
Tedizolid	-	-	-	-	≤0.5	-	-	-	**(29)** **Report only on** *E. faecalis*. **(30)** Breakpoints are based on a dosage regimen of 200 mg administered every 24 h.

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; I, intermediate; **Inv., investigational agent;** MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; R, resistant; S, susceptible; SDD, susceptible-dose dependent; **U, urine;** UTI, urinary tract infection.

Symbols: ^, designation for agents that have the potential to concentrate in the urine; ***, designation for "Other" agents not included in Tables 1 but have established clinical breakpoints.**

Footnotes

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

b. Report only on organisms isolated from the urinary tract.

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.