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

QC Recommendations

Refer to the following:

- Tables 4A-1 and 5A-1 that list acceptable QC ranges applicable for each method
- Appendix I to develop a QC plan

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

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

General Comments

(1) Refer to Table 1D 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 CLSI M02[1]). 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 CLSI M02-Ed14-QG[2]). Hold the Petri dish 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 CLSI M07[3]).

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

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

(5) Synergy between a cell wall–active agent (eg, ampicillin, penicillin, or vancomycin) and an aminoglycoside can be predicted for enterococci by using a high-level aminoglycoside (gentamicin and streptomycin) test (see Table 3L).

(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.

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–β-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–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. **(9)** ***Rx:*** Combination therapy with high-dosage parenteral ampicillin, amoxicillin, penicillin, or vancomycin, plus an aminoglycoside, may be 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. Refer to Table 3L for HLAR testing.
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	**(10)** 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	**(11)** When testing vancomycin against enterococci, plates should be held a full 24 h 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 CLSI 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 3I. See general comment (5) and comment (9).
LIPOGLYCOPEPTIDES									
Dalbavancin	–	–	–	–	≤ 0.25	–	–	–	**(12)** Report only on vancomycin-susceptible *E. faecalis*.
Oritavancin	–	–	–	–	≤ 0.12	–	–	–	See comment (12).
Telavancin	–	–	–	–	≤ 0.25	–	–	–	See comment (12).
Teicoplanin (Inv.)	30 µg	≥ 14	11-13	≤ 10	≤ 8	–	16	≥ 32	

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	
LIPOPEPTIDES									
Daptomycin *E. faecium* only	–	–	–	–	–	≤ 4	–	≥ 8	**(13)** Not routinely reported on organisms isolated from the lower respiratory tract. **(14)** The breakpoint for SDD 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	See comment (13).
MACROLIDES									
Erythromycin*	15 µg	≥ 23	14-22	≤ 13	≤ 0.5	–	1-4	≥ 8	**(15)** Not routinely reported on organisms isolated from the urinary tract.
TETRACYCLINES									
(16) Isolates that test susceptible to tetracycline are considered susceptible to doxycycline and minocycline. Isolates that test intermediate or resistant to tetracycline should be tested against doxycycline or minocycline if those results are needed for treatment.									
Tetracycline (U)[a]	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)[a]	5 µg	≥ 21	16-20^	≤ 15	≤ 1	–	2^	≥ 4	
Levofloxacin (U)[a]	5 µg	≥ 17	14-16^	≤ 13	≤ 2	–	4^	≥ 8	
Gatifloxacin*	5 µg	≥ 18	15-17^	≤ 14	≤ 2	–	4^	≥ 8	
Norfloxacin* (U)[a]	10 µg	≥ 17	13-16	≤ 12	≤ 4	–	8	≥ 16	
NITROFURANS									
Nitrofurantoin (U)[a]	300 µg	≥ 17	15-16	≤ 14	≤ 32	–	64	≥ 128	

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	
ANSAMYCINS									
Rifampin*	5 µg	≥ 20	17-19	≤ 16	≤ 1	–	2	≥ 4	**(17)** ***Rx:*** Rifampin should not be used alone for antimicrobial therapy.
FOSFOMYCINS									
Fosfomycin (U)[a]	200 µg	≥ 16	13-15	≤ 12	≤ 64	–	128	≥ 256	**(18)** Report only on *E. faecalis*. **(19)** 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. **(20)** The 200-µg fosfomycin disk contains 50 µg glucose-6-phosphate.
PHENICOLS									
Chloramphenicol*	30 µg	≥ 18	13-17	≤ 12	≤ 8	–	16	≥ 32	See comment (15).
STREPTOGRAMINS									
Quinupristin-dalfopristin*	15 µg	≥ 19	16-18	≤ 15	≤ 1	–	2	≥ 4	**(21)** Report only on vancomycin-resistant *E. faecium*.
OXAZOLIDINONES									
(22) *E. faecalis* that test susceptible to linezolid are considered susceptible to tedizolid. Isolates that test intermediate or resistant to linezolid should be tested against tedizolid if that result is needed for treatment.									
Linezolid	30 µg	≥ 23	21-22	≤ 20	≤ 2	–	4	≥ 8	
Tedizolid	–	–	–	–	≤ 0.5	–	–	–	See comment (18).

Abbreviations: CAMHB, cation-adjusted Mueller-Hinton broth; h, hour(s); HLAR, high-level aminoglycoside resistance; 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.
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.

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

Footnote

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

References for Table 2D

1 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 14th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2024.

2 CLSI. *M02 Disk Diffusion Reading Guide*. 2nd ed. CLSI quick guide M02-Ed14-QG. Clinical and Laboratory Standards Institute; 2024.

3 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 12th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2024.