

**Table 3K. Test for Detecting High-Level Aminoglycoside Resistance in *Enterococcus* spp.<sup>a</sup> (Includes Disk Diffusion)**

Test	Gentamicin HLAR			Streptomycin HLAR		
	Test method	Broth microdilution	Agar dilution	Disk diffusion	Broth microdilution	Agar dilution
Medium	MHA	BHI <sup>b</sup> broth	BHI <sup>b</sup> agar	MHA	BHI <sup>b</sup> broth	BHI <sup>b</sup> agar
Antimicrobial concentration	120-µg gentamicin disk	Gentamicin, 500 µg/mL	Gentamicin, 500 µg/mL	300-µg streptomycin disk	Streptomycin, 1000 µg/mL	Streptomycin, 2000 µg/mL
Inoculum	Standard disk diffusion procedure	Standard broth dilution procedure	10 µL of a 0.5 McFarland suspension spotted onto agar surface	Standard disk diffusion procedure	Standard broth dilution procedure	10 µL of a 0.5 McFarland suspension spotted onto agar surface
Incubation conditions	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air
Incubation length	16-18 hours	24 hours	24 hours	16-18 hours	24-48 hours (if susceptible at 24 hours, reincubate)	24-48 hours (if susceptible at 24 hours, reincubate)
Results	6 mm = resistant  7-9 mm = inconclusive  ≥ 10 mm = susceptible  MIC correlates: R = > 500 µg/mL S = ≤ 500 µg/mL	Any growth = resistant	> 1 colony = resistant	6 mm = resistant  7-9 mm = inconclusive  ≥ 10 mm = susceptible  MIC correlates: R = > 1000 µg/mL (broth) and > 2000 µg/mL (agar) S = ≤ 1000 µg/mL (broth) and ≤ 2000 µg/mL (agar)	Any growth = resistant	> 1 colony = resistant

**Table 3K. (Continued)**

Test	Gentamicin HLAR			Streptomycin HLAR		
Additional testing and reporting	Resistant: is not synergistic with cell wall-active agent (eg, ampicillin, penicillin, and vancomycin).					
	Susceptible: is synergistic with cell wall-active agent (eg, ampicillin, penicillin, and vancomycin) that is also susceptible.					
	If disk diffusion result is inconclusive: perform an agar dilution or broth dilution MIC test to confirm.					
	Strains of enterococci with ampicillin and penicillin MICs $\geq 16$ $\mu\text{g}/\text{mL}$ are categorized as resistant. However, enterococci with penicillin or ampicillin MICs $> 16$ $\mu\text{g}/\text{mL}$ may be susceptible to synergistic killing by these penicillins in combination with gentamicin or streptomycin (in the absence of high-level resistance to gentamicin or streptomycin, see Subchapter 3.12.2.3 in M07 <sup>1</sup> ) if high doses of penicillin or ampicillin are used. Enterococci possessing higher levels of penicillin (MICs $\geq 128$ $\mu\text{g}/\text{mL}$ ) or ampicillin (MICs $\geq 64$ $\mu\text{g}/\text{mL}$ ) resistance may not be susceptible to the synergistic effect. <sup>2,3</sup> Physicians' requests to determine the actual MIC of penicillin or ampicillin for blood and CSF isolates of enterococci should be considered.					
QC recommendations - routine <sup>c</sup>	<i>E. faecalis</i> ATCC <sup>®d</sup> 29212: 16-23 mm	<i>E. faecalis</i> ATCC <sup>®</sup> 29212 - susceptible	<i>E. faecalis</i> ATCC <sup>®</sup> 29212 - susceptible	<i>E. faecalis</i> ATCC <sup>®</sup> 29212: 14-20 mm	<i>E. faecalis</i> ATCC <sup>®</sup> 29212 - susceptible	<i>E. faecalis</i> ATCC <sup>®</sup> 29212 - susceptible
QC recommendations - lot/shipment <sup>e</sup>		<i>E. faecalis</i> ATCC <sup>®</sup> 51299 - resistant	<i>E. faecalis</i> ATCC <sup>®</sup> 51299 - resistant		<i>E. faecalis</i> ATCC <sup>®</sup> 51299 - resistant	<i>E. faecalis</i> ATCC <sup>®</sup> 51299 - resistant

Abbreviations: ATCC<sup>®</sup>, American Type Culture Collection; BHI, brain heart infusion; CSF, cerebrospinal fluid; HLAR, high-level aminoglycoside resistance; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control.

**Footnotes**

- a. Other aminoglycosides do not need to be tested, because their activities against enterococci are not superior to gentamicin and streptomycin.
- b. BHI: Even though not as widely available, dextrose phosphate agar and broth have been shown in limited testing to perform comparably.
- c. QC recommendations - routine

Test negative (susceptible) QC strain:

- With each new lot/shipment of testing materials
- Weekly if the test is performed at least once a week and criteria for converting from daily to weekly QC testing have been met (see Subchapter 4.7.2.3 in M02<sup>4</sup> and M07<sup>1</sup>)
- Daily if the test is performed less than once per week and/or if criteria for converting from daily to weekly QC testing have not been met

**Table 3K. (Continued)**

- d. ATCC® is a registered trademark of the American Type Culture Collection.
- e. QC recommendations - lot/shipment  
Test positive (resistant) QC strain at minimum with each new lot/shipment of testing materials.

**References for Table 3K**

- 1 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2018.
- 2 Torres C, Tenorio C, Lantero M, Gastañares MJ, Baquero F. High-level penicillin resistance and penicillin-gentamicin synergy in *Enterococcus faecium*. *Antimicrob Agents Chemother*. 1993;37(11):2427-2431.
- 3 Murray BE. Vancomycin-resistant enterococci. *Am J Med*. 1997;102(3):284-293.
- 4 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.