

Table 3K. Test for Detecting High-Level Aminoglycoside Resistance in *Enterococcus* spp.^a (includes disk diffusion)

| Test | Gentamicin HLAR | | | Streptomycin HLAR | | |
|-----------------------------|---|-----------------------------------|---|---|--|---|
| | Disk diffusion | Broth microdilution | Agar dilution | Disk diffusion | Broth microdilution | Agar dilution |
| Test method | Disk diffusion | Broth microdilution | Agar dilution | Disk diffusion | Broth microdilution | Agar dilution |
| Medium | MHA | BHI ^b broth | BHI ^b agar | MHA | BHI ^b broth | BHI ^b 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 ≥ 16 $\mu\text{g}/\text{mL}$ are categorized as resistant. However, enterococci with penicillin MICs ≤ 64 $\mu\text{g}/\text{mL}$ or ampicillin MICs ≤ 32 $\mu\text{g}/\text{mL}$ 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 ¹) if high doses of penicillin or ampicillin are used. Enterococci possessing higher levels of penicillin (MICs ≥ 128 $\mu\text{g}/\text{mL}$) or ampicillin (MICs ≥ 64 $\mu\text{g}/\text{mL}$) resistance may not be susceptible to the synergistic effect. ^{2,3} Physicians' requests to determine the actual MIC of penicillin or ampicillin for blood and CSF isolates of enterococci should be considered. | | | | | |
| QC recommendations - routine ^c | <i>E. faecalis</i> ATCC ^{®d} 29212: 16-23 mm | <i>E. faecalis</i> ATCC [®] 29212 - susceptible | <i>E. faecalis</i> ATCC [®] 29212 - susceptible | <i>E. faecalis</i> ATCC [®] 29212: 14-20 mm | <i>E. faecalis</i> ATCC [®] 29212 - susceptible | <i>E. faecalis</i> ATCC [®] 29212 - susceptible |
| QC recommendations - lot/shipment ^e | | <i>E. faecalis</i> ATCC [®] 51299 - resistant | <i>E. faecalis</i> ATCC [®] 51299 - resistant | | <i>E. faecalis</i> ATCC [®] 51299 - resistant | <i>E. faecalis</i> ATCC [®] 51299 - resistant |

Abbreviations: ATCC[®], 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

- Other aminoglycosides do not need to be tested, because their activities against enterococci are not superior to gentamicin and streptomycin.
- Even though not as widely available, dextrose phosphate agar and broth have been shown in limited testing to perform comparably with BHI media.
- 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 M02⁴ and M07¹)
- 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.