

SCREENING RESISTANCE FOR HIGH-LEVEL AMINOGLYCOSIDE RESISTANCE (HLAR) AND VANCOMYCIN RESISTANCE IN *ENTEROCOCCUS* SPP.

Screen Test	Gentamicin HLAR	
Test Method	Disk Diffusion	Agar Dilution
Medium	Mueller Hinton Agar	BHI ^a Agar
Antimicrobial Concentration	120ug Gentamicin Disk	Gentamicin 500ug/ml
Inoculum	Standard disk diffusion recommendations	10ul of a 0.5 McFarland suspension spotted onto agar surface
Incubation Conditions	35 +/- 2 degrees C.; ambient air	35 +/- 2 degrees C.; ambient air
Incubation Length	16-18 hours	24 hours
Results	6mm = Resistant; 7-9mm = Inconclusive; ≥10mm = Susceptible.	>1 colony = Resistant
QC Recommendations	<i>E. faecalis</i> ATCC [®] 29212: 16-23mm	<i>E. faecalis</i> - Susceptible ATCC [®] 29212 <i>E. faecalis</i> - Resistant ATCC [®] 51299
Further Testing and Reporting	Resistant: is not synergistic with cell-wall-active agent (eg, ampicillin, penicillin, and vancomycin). Susceptible: is synergistic with cell-wall-active agents (eg, ampicillin, penicillin, and vancomycin) that is also susceptible. If disk diffusion result is inconclusive: perform an agar dilution or broth microdilution test to confirm.	

Screen Test	Streptomycin HLAR		Vancomycin Resistance
Test Method	Disk Diffusion	Agar Dilution	Agar Dilution
Medium	Mueller Hinton Agar	BHI ^a Agar	BHI ^a Agar
Antimicrobial Concentration	300ug Streptomycin Disk	Streptomycin, 2000ug/ml	Vancomycin, 6ug/ml
Inoculum	Standard disk diffusion recommendations	10ul of a 0.5 McFarland suspension spotted onto agar surface	1-10ul of a 0.5 McFarland suspension spotted onto agar surface
Incubation Conditions	35 +/- 2 degrees C.; ambient air	35 +/- 2 degrees C.; ambient air	35 +/- 2 degrees C.; ambient air
Incubation Length	16-18 hours	24-48 hours (if susceptible at 24 hours, reincubate)	24 hours
Results	6mm = Resistant; 7-9mm = Inconclusive; ≥10mm = Susceptible.	>1 colony = Resistant	>1 colony = presumptive vancomycin resistance
QC Recommendations	<i>E. faecalis</i> - ATCC [®] 29212: 14-20mm	<i>E. faecalis</i> - Susceptible ATCC [®] 29212 <i>E. faecalis</i> - Resistant ATCC [®] 51299	<i>E. faecalis</i> - Susceptible ATCC [®] 29212 <i>E. faecalis</i> - Resistant ATCC [®] 51299
Further Testing and Reporting	Resistant: is not synergistic with cell-wall-active agent (eg, ampicillin, penicillin, and vancomycin). Susceptible: is synergistic with cell-wall-active agents (eg, ampicillin, penicillin, and vancomycin) that is also susceptible. If disk diffusion result is inconclusive: perform an agar dilution or broth microdilution test to confirm.		Perform vancomycin MIC and tests for motility and pigment production to distinguish species with acquired resistance (<i>VanA</i> and <i>VanB</i>) from those with intrinsic, intermediate-level resistance to vancomycin (<i>VanC</i>) such as <i>E. gallinarum</i> and <i>E. casseliflavus</i> , which often grow on the vancomycin screen plate. In contrast to other enterococci, <i>E. casseliflavus</i> and <i>E. gallinarum</i> with vancomycin MICs or 8-16ug/ml (intermediate) differ from vancomycin-resistant enterococci (VRE) for infection control purposes.

Footnote

- a. BHI = Brain Heart Infusion; even though not as widely available, Dextrose Phosphate Agar and Broth have been shown in limited testing to perform comparably.

REFERENCES

Performance Standards for Antimicrobial Disk Susceptibility Tests; Informational Supplement, M100. 2009. Clinical Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

Methods for Dilution Antimicrobial Test for Bacteria that Grow Aerobically, M7. 2009. Clinical Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

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