PROCEDURE: SYNERGY QUAD PLATE

I. PRINCIPLE

Brain Heart Infusion (BHI) Agar is recommended for HLR screening of *Enterococcus* spp. by the Clinical and Laboratory Standards Institute (CSLI). BHI Agar contains brain heart infusion, peptones, and dextrose which provide nitrogen, carbon, sulfur, vitamins, and a carbohydrate source. Gentamicin (500 ug/ml) and streptomycin (2000 ug/ml) are added to detect high-level aminoglycoside resistance, and vancomycin (6 ug/ml) is included to detect vancomycin resistance. Agar is a solidifying agent. Amaranth is a nontoxic food dye which provides a distinguishing pink color to the control quadrant.

II. STORAGE AND HANDLING

A. This product is ready for use and no further preparation is necessary.
B. The product should be stored in its original container at 2-8 °C until used.
C. Allow product to come to room temperature before use.
D. Do not incubate prior to use.

III. MATERIALS NEEDED

A. Synergy Quad plate
B. 5 mL 0.85% Saline
C. Sterile inoculating loops
D. 10 uL pipette
E. Pipette tips

IV. QUALITY CONTROL

A. *Enterococcus faecalis* ATCC 29212 – Growth in quadrant I, no growth in quadrant II, III, IV
    1. Quadrant IV (streptomycin) incubated an additional 24 hours
B. *Enterococcus faecalis* ATCC 51299 – Growth in quadrant I, II, III, IV

V. TEST PROCEDURE

A. Obtain a pure 18-24 hour culture of *Enterococcus.*
B. Prepare a broth suspension of the culture and adjust to the turbidity of a McFarland 0.5 Standard or equivalent.
C. Spot inoculate each quadrant of the plate with 10µl of a 0.5 MacFarland suspension. Inoculate the vancomycin quadrant using either a 1 uL or 10 uL calibrated loop. Spread inoculum over a 10-15 mm area.
D. Allow inoculum to absorb into the agar surface.
E. Invert plates and incubate ambiently at 35-37 °C for a full 24 hours.
F. At 24 hours, plates with no growth in Quadrant IV (streptomycin) should be reincubated for an additional 24 hours, then re-examined.

VI. INTERPRETATION

QUADRANT I Control – Clear	Growth demonstrates viability of the test isolate broth suspension. If no growth is evident at 24 hours the test is invalid and must be repeated.
QUADRANT II 500 µg/ml Gentamicin – Red	Growth – Synergy cannot be predicted No Growth – Synergy can be predicted
QUADRANT III 6 µg/ml Vancomycin – Yellow	*Growth – Synergy cannot be predicted No Growth – Synergy can be predicted
QUADRANT IV 2000 µg/ml Streptomycin – Blue	Growth – Synergy cannot be predicted No Growth – Synergy can be predicted

*Streptomycin quadrant incubated an additional 24 hours

VII. PRECAUTIONS

A. This product is "For In-Vitro Diagnostic Use" and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after their use. Directions should be read and followed carefully.
B. This product should not be used if any of the following apply:
    1. There is evidence of dehydration
    2. Product is contaminated
    3. Media color has changed
    4. Media is expired
    5. There are other signs of deterioration

VIII. LIMITATIONS

A. This product is a screening system for use in predicting the synergistic contribution of an aminoglycoside and vancomycin when testing enterococcal isolates requiring combination therapy. Definitive methods for determining synergy include the checkerboard technique and time-kill tests.
B. This product is intended to be used with *Enterococcus* species isolates only. Performance characteristics for other organisms have not been established.
C. Enterococci may be resistant to penicillin and ampicillin due to production of penicillin-binding proteins or beta lactamase. Consult current CSLI standard for appropriate test methodology.

IX. REFERENCES

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E. Balows A, Haulser WJ, Hermann, KL, Isenberg HD and Shadomy, HJ. Man. of Clin. Microbiol. 5th Ed, Amer. Med. Soc. for Microbiology, 1991.
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X. REVISIONS

G. 06/17/2024 Fixed principle, updated test procedure and limitations, general formatting improvements