

# BD BBL™ Prepared Plated Medium for the Detection of Enterococci with High-Level Aminoglycoside and Vancomycin Resistance

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## Enterococcus Screen Agar QUAD Plate with Streptomycin / Gentamicin / Vancomycin

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### INTENDED USE

Enterococcus Screen Agar is used to test enterococci for high level resistance to aminoglycosides and vancomycin to predict the synergistic activity of these antimicrobials.

### SUMMARY AND EXPLANATION

Enterococci are known to cause a wide variety of infections. Most commonly they infect the urinary tract, abdomen, bloodstream, endocardium, biliary tract, burn wounds and indwelling catheters.<sup>1</sup> *Enterococcus faecalis* causes 80 to 90% of infections, while *E. faecium* causes the majority of the remainder.<sup>2</sup> Today the enterococci are the fourth leading cause of bacteremia in the United States.<sup>3</sup> The case/fatality rates for enterococcal bacteremia range from 12 to 68% with death due to sepsis in 4 to 50% of the cases.<sup>4</sup>

Treatment of enterococcal infections with either penicillin or vancomycin alone fails to kill enterococci resulting in relapse of infection.<sup>5</sup> Enterococci for years were known to have low intrinsic resistance to a variety of  $\beta$ -lactam as well as aminoglycoside antibiotics.<sup>6</sup> The addition of an aminoglycoside to which the isolate has demonstrated susceptibility results in both *in vitro* and *in vivo* synergism producing a bactericidal effect.<sup>7</sup> This synergistic effect is thought to be due to the penicillin or vancomycin damaging the integrity of the cell wall, thus allowing the aminoglycoside to penetrate and inhibit bacterial protein synthesis.<sup>8</sup> The emergence of high level resistance to streptomycin ( $\geq 2000 \mu\text{g/mL}$ ), gentamicin ( $\geq 500 \mu\text{g/mL}$ ) and vancomycin ( $\geq 6 \mu\text{g/mL}$ ) results in the failure of the penicillin or vancomycin-aminoglycoside combinations to eradicate the infecting organisms. Therefore, testing for high level resistance to streptomycin, gentamicin and vancomycin is important. The use of a Brain Heart Infusion Agar (BHIA) containing streptomycin (2000  $\mu\text{g/mL}$ ), gentamicin (500  $\mu\text{g/mL}$ ), or vancomycin (6  $\mu\text{g/mL}$ ) is recommended by the Clinical and Laboratory Standards Institute (CLSI) for testing high level resistance.<sup>9</sup>

### PRINCIPLES OF THE PROCEDURE

Brain Heart Infusion Agar is a general-purpose medium suitable for the cultivation of a wide variety of microorganisms and is recommended for agar screen susceptibility testing of enterococci.<sup>9</sup>

The meat infusion solids and peptones are sources of organic nitrogen, carbon, sulfur, vitamins, and trace substances. Dextrose is the carbohydrate source. The medium is buffered through the use of disodium phosphate. Streptomycin at 2000  $\mu\text{g/mL}$  and gentamicin at 500  $\mu\text{g/mL}$  are used to detect high level aminoglycoside resistance.<sup>9</sup> Vancomycin at 6  $\mu\text{g/mL}$  is used to detect resistance to vancomycin.<sup>9</sup> The Food, Drug & Cosmetic (FD&C) dyes are inert and added for easy visual identification of the antimicrobials.

### REAGENTS

#### Enterococcus Screen Agar

Brain Heart Infusion Agar (BHIA)

Approximate Formula\* Per Liter Purified Water

Brain Heart, Infusion from (solids).....	8.0 g
Peptic Digest of Animal Tissue.....	5.0 g
Pancreatic Digest of Casein.....	16.0 g
Sodium Chloride.....	5.0 g
Dextrose.....	2.0 g
Disodium Phosphate.....	2.5 g
Agar.....	13.5 g

**Section I** consists of BHIA (Growth Control)

**Section II** consists of BHIA with 0.5 g/L gentamicin and 1.02 g/L FD&C Red #40 dye.

**Section III** consists of BHIA with 6.0 mg/L vancomycin and 0.56 g/L FD&C Yellow #5 dye.

**Section IV** consists of BHIA with 2.0 g/L streptomycin and 0.4 g/L FD&C Blue #1 dye.

\* Adjusted and/or supplemented as required to meet performance criteria.

#### Warnings and Precautions

For *in vitro* Diagnostic Use.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding. If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

**Storage Instructions:** On receipt, store plates in the dark at 2 to 8 °C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2 to 8 °C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

**Product Deterioration:** Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

### SPECIMEN COLLECTION AND HANDLING

These plates are not intended for use with specimens or mixed cultures. The organism to be tested must first be in pure culture and presumptively identified as *Enterococcus* species.

### PROCEDURE

**Material Provided:** Enterococcus Screen Agar.

**Materials Required But Not Provided:** Ancillary culture media, reagents, quality control organisms and laboratory equipment as required for this procedure.

### Test Procedure

1. Prepare the inoculum by suspending several well-isolated colonies of the enterococcal isolate from an 18 to 24 h plate culture into a tube of **Trypticase™** Soy Broth and adjust the turbidity to be equivalent to a 0.5 McFarland turbidity standard.
2. Spot inoculate each section of the plate with 10  $\mu\text{L}$  of the adjusted suspension.<sup>10</sup>
3. Allow the inoculum spots to absorb in the agar surfaces.
4. Incubate plates at  $35 \pm 2$  °C aerobically for a full 24 h. If negative at 24 h, reincubate streptomycin tests an additional 24 h.

### User Quality Control:

1. Examine plates for signs of deterioration as described under "Product Deterioration."
2. Check performance by inoculating a representative sample of plates with pure cultures of stable control organisms that produce known, desired reactions. The following cultures are recommended:

TEST STRAINS	EXPECTED RESULTS				
	Section	I	II	III	IV*
<i>Enterococcus faecalis</i> ATCC™ 29212		+	–	–	–
<i>Enterococcus faecalis</i> ATCC 51299		+	+	+	+
<i>Enterococcus gallinarum</i> ATCC 49573		+	–	+	–

+ = Growth > one colony

– = No growth or one colony

\* If section IV is negative at 24 h, reincubate for another 24 h.

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI guidance and CLIA regulations for appropriate Quality Control practices.

### RESULTS

Following a full 24 h of incubation, observe plates for growth. Growth on Section I (BHIA Control) indicates viable test organisms in the inoculum broth suspension and the test is valid. If there is no growth, the test is invalid and must be repeated.

Growth on:

Section II – Red (BHIA with gentamicin) and/or

Section III – Yellow (BHIA with vancomycin) and/or

Section IV – Blue (BHIA with streptomycin)

indicates that the antimicrobial would not be synergistic in combination therapy. No growth indicates synergy may be predicted.

### LIMITATIONS OF THE PROCEDURE

This product is intended for use with *Enterococcus* species. Occasionally enterococcal isolates with borderline antimicrobial susceptible MICs may show growth. This product provides a screening method for predicting synergistic contributions of the aminoglycosides with penicillin or vancomycin. The synergistic contribution of other aminoglycosides cannot be predicted from streptomycin or gentamicin results nor can the synergistic contribution of penicillin be predicted from the vancomycin results.

The checkerboard technique and time-kill tests are definitive methods for determining synergy.<sup>10</sup>

The enterococcal test strain may be resistant to penicillin and ampicillin from the alteration of penicillin-binding proteins or the production of  $\beta$ -lactamase. Consult the CLSI document for appropriate test methods.<sup>9</sup>

### PERFORMANCE CHARACTERISTICS

The agar screen test procedure for detecting high-level aminoglycoside and vancomycin resistant enterococci recommended by CLSI was performed in-house with 49 *Enterococcus* isolates using **BBL™** screen media that contained BHIA with 500  $\mu\text{g/mL}$  gentamicin, 6  $\mu\text{g/mL}$  vancomycin, and 2000  $\mu\text{g/mL}$  streptomycin. The 49 enterococcus isolates consisted of 21 *E. faecalis*, 18 *E. faecium*, 4 *E. gallinarum*, 2 *E. raffinosus*, 1 *E. casseliflavus*, 1 *E. mundtii*, and 2 *E. avium*. The resistance patterns for the 49 enterococci are shown in Table 1.

**Table 1. In-House Study**

(% correlation to expected results)

	Total No. of Strains Tested	No. of Tests Resistant at 24 hours/Expected Results	No. of Tests Resistant at 48 hours/Expected Results
Gentamicin	49	29/29 (100%)	
Vancomycin	49	27/27 (100%)	
Streptomycin	49	24/26 (92%)	26/26 (100%)

Phenotypic characterization included the use of agar and/or broth dilution to establish gentamicin, vancomycin and streptomycin MICs. Of the 49 isolates, all gave 100% correlation between the test results and expected results.

Reproducibility studies (3x/day for 3 days) were done at two field sites with 14 enterococcal isolates. The 14 isolates consisted of 7 *E. faecalis*, 3 *E. faecium*, 1 *E. gallinarum*, 1 *E. avium*, 1 *E. casseliflavus*, and 1 *E. raffinosus*. The resistance patterns for the 14 enterococci are shown in Table 2.

**Table 2. Field Site Reproducibility Study**  
(% correlation to expected results)

	Total No. of Strains Tested	Total No. of Tests	No. of Tests Resistant at 24 hours/Expected Results
Gentamicin	14	279	162/162 (100%)
Vancomycin	14	279	135/135 (100%)
Streptomycin	14	279	160/162 (99%)

Phenotypic characterization included the use of agar and/or broth dilution to establish gentamicin, vancomycin and streptomycin MICs. Here there was also 100% correlation between the test results and expected results.

AVAILABILITY

Cat. No. Description

222201 **BBL™** Enterococcus Screen Agar QUAD Plate with Streptomycin / Gentamicin / Vancomycin, Pkg. of 10 plates.

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