remel

BRAIN HEART INFUSION AGAR w/ VANCOMYCIN 6 µg/ml

INTENDED USE

Remel Brain Heart Infusion (BHI) Agar w/ Vancomycin is a solid medium recommended for use in qualitative procedures as an agar screen test for use in the detection of vancomycin resistance in enterococci.

SUMMARY AND EXPLANATION

Since first detected in 1986, vancomycin-resistant enterococci are now recovered with increasing frequency.[1] Various phenotypes responsible for acquired resistance in enterococci have been described with high to low level vancomycin resistance and cross-resistance to teicoplanin.[2] Detection has been difficult due to failure of commonly used susceptibility methods to identify those isolates with low-level resistance.[3] An agar screen test with BHI Agar supplemented with 6 µg/ml of vancomycin has been recommended by the Clinical and Laboratory Standards Institute (CLSI) as a reliable method for the detection of all phenotypes of vancomycin-resistant enterococci.[4,5]

PRINCIPLE

The recommended medium for agar screening is BHI Agar. This medium contains meat infusion, peptone, and dextrose which provide nitrogen, carbon, sulfur, vitamins, and a carbohydrate source. Agar is a solidifying agent. BHI Agar is supplemented with 6 µg/ml of vancomycin to detect resistant strains of *Enterococcus* spp.

REAGENTS (CLASSICAL FORMULA)*

Casein Peptone	14.5 g
Meat Peptone	7.0 g
Brain Heart Infusion Solids	6.0 g
Sodium Chloride	5.0 g
Disodium Phosphate	2.5 g
Dextrose	2.0 g
Vancomycin	6.0 mg
Agar	15.0 g
Demineralized Water	1000.0 ml

pH 7.4 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PRECAUTIONS

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 use. Directions should be read and followed carefully.

STORAGE

This product is ready for use and no further preparation is necessary. Store product in its original container at 2-8°C until used. Allow product to equilibrate to room temperature before use. Do not incubate prior to use.

PRODUCT DETERIORATION

This product should not be used if (1) there is evidence of dehydration, (2) the product is contaminated, (3) the color has changed, (4) the expiration date has passed, or (5) there are other signs of deterioration.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORT

Specimens should be collected and handled following recommended guidelines.[7]

MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Loop sterilization device, (2) Inoculating loop, swabs, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental media, (5) Quality control organisms, (6) McFarland 0.5 standard or equivalent (REF R20410), photometric device, (7) Micropipettor, calibrated loop, or Steers replicator.[8]

PROCEDURE[4,5]

1. Obtain a pure, 18-24 hour old culture of enterococci.
2. Prepare a suspension of the culture and adjust to the turbidity of a McFarland 0.5 standard or equivalent visually or by utilizing a photometric device.
3. Inoculate the plate with the adjusted organism suspension using either a 1 µl or 10 µl calibrated loop.
4. Spread the inoculum over a 10-15 mm area and allow to absorb into the agar surface.
5. Invert the plate and incubate in ambient air at 35 ± 2°C for a full 24 hours prior to interpretation.
6. After incubation, examine plate for evidence of small colonies (>1 colony) or a film of growth.

INTERPRETATION OF THE TEST

Growth - Resistant (good growth, light growth, or >1 individual colony)

No growth - Susceptible

QUALITY CONTROL

All lot numbers of BHI Agar w/ Vancomycin have been tested using the following quality control organisms and have been found to be acceptable. This quality control testing conforms with or exceeds CLSI standards.[4-6] Controls should be included each time a susceptibility test is performed or weekly if satisfactory performance can be documented according to CLSI standards. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
**Enterococcus faecalis* ATCC® 29212	Ambient, 24 h @ 35 ± 2°C	No growth
**Enterococcus faecalis* ATCC® 51299	Ambient, 24 h @ 35 ± 2°C	Growth

*CLSI recommended organism

LIMITATIONS

1. This product is a screening system for use in the detection of vancomycin-resistant enterococci. Data is not available for other organisms.
2. A blood agar plate may be used as a growth control to monitor organism viability and purity.

PERFORMANCE CHARACTERISTICS[9]

BHI Agar w/ Vancomycin was evaluated using 105 strains of *Enterococcus* (59 *E. faecalis,* 24 *E. faecium,* 13 *E. gallinarum,* and 9 *E. casseliflavus*). The phenotype was established by Mueller Hinton agar dilution and the genotype by PCR and/or Southern hybridization technology (characteristics of these strains are listed in Table 1). For this analysis, vancomycin susceptibility was defined as isolates with an MIC value of ≤4 µg/ml. Resistance included both intermediate (MIC 8-16 µg/ml) and resistant (MIC ≥ 32 µg/ml) isolates.

TABLE 1:
Summary of Enterococcal Strains Used for Vancomycin Evaluation

Susceptibility Category[a]	Species	No. of strains	Vancomycin MIC range (µg/ml)	Genotypic Profile[b]
Susceptible	*E. faecalis*	42	≥0.5-4	-
	E. faecium	5	4	-
	E. gallinarum	2	1-4	C, 1
	E. casseliflavus	7	2-4	ND
Intermediate	*E. faecalis*	3	16	B, 2
	E. faecium	0	NA	-
	E. gallinarum	11	8-16	C,11
	E. casseliflavus	2	8	ND
Resistant	*E. faecalis*	14	32->512	A,1; B,13
	E. faecium	19	128->512	A,5; B,14
	E. gallinarum	0	NA	-
	E. casseliflavus	0	NA	-
	Total	105		

[a]As determined by agar (Mueller Hinton) dilution: susceptible, ≤4 µg/ml; intermediate, 8-16 µg/ml; resistant, ≥32 µg/ml.

[b]Based on PCR and/or Southern hybridization studies, strains were classified as not susceptible due to *van*A- (A), *van*B- (B), or *van*C- (C) mediated resistance; for *E. casseliflavus,* analysis for the presence of the *van*C1 gene was not available (ND); (-), no gene detected.

TABLE 2: Summary of Results

Strain Profile	Inoculum	Incubation	No. (%) Correct Results	
			All Strains	*E. faecalis* and *E. faecium* only
Vancomycin Susceptible (56)	10^5	24 hours	52 (93)[a]	46/47 (98)
	10^6	24 hours	51 (91)[b]	46/47 (98)
Vancomycin Resistant (49)	10^5	24 hours	49 (100)	36/36 (100)
	10^6	24 hours	49 (100)	36/36 (100)

[a]Discordant results at 10^5 inoculum: one phenotypically susceptible *E. faecalis* (MIC = 4 µg/ml, Etest® MIC = 6 µg/ml), two phenotypically susceptible *E. casseliflavus* (MICs of 4 and 8 µg/ml on Mueller Hinton and BHI Agar, respectively), and one *vanC* negative, phenotypically susceptible *E. gallinarum*.

[b]Discordant results at 10^6 inoculum: as stated above with the exception of one phenotypically susceptible *E. casseliflavus* (MICs of 4 and 8 µg/ml on Mueller Hinton and BHI Agar, respectively).

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PACKAGING

Brain Heart Infusion Agar w/ Vancomycin 6 µg/ml:
REF R01176, 13 X 85 mm Plate 10/Pk

Symbol Legend

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)

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