IFU

Instructions for Use

BRAIN HEART INFUSION AGAR (BHIA) WITH VANCOMYCIN

Cat. no. G14	BHIA with Vancomycin, 15x100mm Plate, 18ml	10 plates/bag

INTENDED USE

Hardy Diagnostics Brain Heart Infusion Agar (BHIA) with 6µg/ml of vancomycin is screening method for detection of vancomycin-resistant enterococci from pure cultures.[8]

SUMMARY

Vancomycin-resistant enterococci were first recognized in Europe and the United States in the late 1980's.[11,12] Since then, the incidence of resistance has increased from 0.3% in 1989 to 7.9% in 1993.[13] Automated and disk diffusion methods may fail to detect resistant strains. Tenover, et al. found, with moderate and low-level resistance, automated methods had high levels of incorrect results.[14] In evaluating the disk diffusion method, Swenson, et al. found 14.5% minor errors and 2.2% very major errors. [10] In 1992 Willey, et al. described an agar screening method for detection of vancomycin-resistant enterococci; this medium was found to be 100% sensitive and specific.[9] Optimum conditions for inoculum size, medium, drug concentration, and length of incubation were established by Swenson et al.[5] BHIA with 6µg/ml vancomycin is recommended by the CLSI (Clinical and Laboratory Standards Institute - formerly NCCLS) for the detection of vancomycin-resistant enterococci.[8] If the organism does not grow on this medium, it is considered susceptible; if the organism does grow, it is considered resistant to vancomycin.

FORMULA

Ingredients per liter of deionized water:*

Beef Heart Infusion	250.0gm
Calf Brain Infusion	200.0gm
Proteose Peptone	10.0gm
Sodium Chloride	5.0gm
Disodium Phosphate	2.5gm
Dextrose	2.0gm
Vancomycin	6.0mg
Agar	15.0gm

Final pH 7.4 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Specimen Collection: This product is intended for use as a screening medium for the detection of vancomycin resistance enterococci from pure cultures.[8] Information on specimen collection may be found in standard references on the subject.[6] Information on specimen processing may be found in these references and in appropriate package inserts on isolation media.

The performance of this medium for vancomycin resistance testing is dependent on a properly prepared inoculum. The organism should be in pure culture and 18 to 24 hours old.

Method of Use:

1. Using a sterile inoculating loop, select 3 to 4 well isolated colonies from a non-selective agar plate such as Tryptic Soy Agar (TSA) or Blood Agar, 5% (Cat. no. G60 and A10, respectively). The organism should be in pure culture and 18 to 24 hours old.

2. Prepare a suspension in saline or Tryptic Soy Broth (TSB) equivalent to a 0.5 McFarland standard.

3. Using a 1ul or 10µl loop, spot inoculate the surface of the agar medium.[8,15]

4. Allow the plates to stand at room temperature until the moisture in the inoculum spots has been absorbed into the agar (i.e. until the spots are dry).

5. Invert the plates and incubate aerobically at 35ºC. for a full 24 hours.[8,5]

INTERPRETATION OF RESULTS

Growth of >1 colony indicates a vancomycin-resistant organism; no growth indicates that the organism is susceptible.

Screening tests for vancomycin-resistant enterococci have been shown to be comparable in reliability to standard methods in detecting clinically significant resistance, and additional confirmatory tests are unnecessary.[8]

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

The test organism must be in pure culture and about 24 hours old. The test procedure must be followed exactly.

The density of the organism suspension is critical, as lighter or heavier suspensions may cause erroneous results.

The CLSI recommends a full 24 hour incubation at 35ºC. before reading.[7]

E. gallinarum/*casseliflavus* are considered intrinsically resistant due to the presence of the VanC gene which may not be expressed when testing on the vancomycin screen plate.

BHIA with Vancomycin is for use in detection of vancomycin-resistant enterococci from colonies grown on solid media. Direct specimen testing is not recommended.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as inoculating loops, slides, staining supplies, other culture media, McFarland standard, microscopes, incinerators, refrigerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Enterococcus faecalis ATCC® 51299	A**	24hr	35°C	Aerobic	Growth (vancomycin-resistant)
Enterococcus faecalis ATCC® 29212	A**	24hr	35°C	Aerobic	Inhibition (vancomycin-sensitive)

* Refer to the document "Inoculation Procedures for Media QC" for more information.

****Note:** Turbidity of inoculum is matched to a 0.5 McFarland standard. No further dilution is made. A 1µl or 10µl is spot inoculated onto the agar surface. Results are read at 24 hours.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

BHIA with Vancomycin should appear translucent, and light amber in color.

PERFORMANCE CHARACTERISTIC

In a study at two independent test sites, 80 organisms including 37 *E. faecium*, 19 *E. faecalis*, 11 *E. gallinarum*, and 13 *E. casseliflavus* were tested according to the CLSI recommended procedure for screening of vancomycin resistance of enterococci.[8] The breakdown for the test organism MIC's were as follows; 21 isolates had an MIC $\leq$4, 12 isolates between 8 and 16. Forty-seven (47) isolates had MIC's $\geq$32. Isolates with MIC $\leq$4 were considered susceptible; MIC $\geq$ 8 were considered resistant. Study results showed 95% correlation (152/160) with six of the eight (6/8) discrepancies due to the *E. gallinarum*/*casseliflavus* group. The *E. gallinarum*/*casseliflavus* group is intrinsically resistant due to the VanC gene. Resistance due to the VanC gene is difficult to determine but is usually in the intermediate range when testing by a MIC reference method. No growth problems were encountered in the study.

REFERENCES

1. Anderson, N.L., et al. 2005. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

2. Forbes, B.A., et al. 2007. *Bailey and Scott's Diagnostic Microbiology*, 12th ed. C.V. Mosby Company, St. Louis, MO.

3. Facklam, R.R., and M.D. 1970. Moody. *Appl. Microbiol.*; 20:245.

4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

5. Swenson, J.M., et al. 1994. Development of a standardized screening method for detection of vancomycin-resistant enterococci. *J. Clin. Microbiol.*; 32:1700-1704.

6. Murray, P.R., et al. 2007. *Manual of Clinical Microbiology*, 9th ed. American Society for Microbiology, Washington, D.C.

7. *Performance Standards for Antimicrobial Susceptibility Testing*, M100. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

8. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*, M7. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA. 1993.

9. Willey, B.M., et al. 1992. Detection of vancomycin resistance in *Enterococcus* species. *J. Clin. Microbiol.*; 30:1621-1624.

10. Swenson, J.J., et al. 1992. New vancomycin disk diffusion breakpoints for enterococci. The National Committee for Clinical Laboratory Standards Working Group on Enterococci and F.C. Tenover. *J. Clin. Microbiol.*; 30:2525-2528.

11. Uttley, et al. 1988. *Vancomycin-resistant enterococci*. Lancet i:57-58. (Letter).

12. Leclercq, et al. 1988. Plasmid-mediated resistance to vancomycin and teicoplanin in *Enterococcus faecium*. *N. Engle. J. Med.*; 319:157-161.

13. Centers for Disease Control, 1993. *Nosocomial enterococci resistant to vancomycin*. United States, 1989-1993.

14. Tenover, F.C., et al. 1993. Ability of clinical laboratories to detect antimicrobial agent-resistant enterococci. *J. Clin. Microbiol.*; 31:1695-1699.

15. Van Horn, K.G., et al. 1996. Evaluation of commercial vancomycin agar screen plates for detection of vancomycin-resistant enterococci. *J. Clin. Microbiol.*; 34:2042-2044.

16. Tenover, F.C., et al. 1995. Ability of commercial and reference antimicrobial susceptibility testing methods to detect vancomycin-resistant enterococci. *J. Clin. Microbiol.*; 33:1524-1527.

17. Jorgensen, J.H., et al. 1996. Comparison of inoculation methods for testing enterococci by using vancomycin screening agar. *Journal of Clin. Micro.*; Vol. 34, No. 11, pp. 2841-2842.

IFU-10053[B]

1430 West McCoy Lane, Santa Maria, CA 93455, USA
Phone: (805) 346-2766 ext. 5658
Fax: (805) 346-2760
Website: HardyDiagnostics.com
Email: TechnicalServices@HardyDiagnostics.com
Ordering Information

Distribution Centers:
California · Washington · Utah · Arizona · Texas · Ohio · New York · Florida · North Carolina

The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207F [D]