HardyCHROM™ VRE

Cat. no. G333	HardyCHROM™ VRE Agar, 15x100mm Plate, 18ml	10 plates/bag
Cat. no. P19	HardyCHROM™ VRE Agar, 15x60mm Contact Plate, 15ml	10 plates/bag

INTENDED USE

Hardy Diagnostics HardyCHROM™ VRE is used as a cost effective primary screening medium for the detection and differentiation of vancomycin-resistant enterococci (VRE), such as *Enterococcus faecalis* and *E. faecium*, from fecal and rectal cultures, by using chromogenic substances.

SUMMARY

Vancomycin is a glycopeptide antibiotic that is crucial for the treatment of penicillin- and aminoglycoside-resistant enterococci infections.[1] Recognition of vancomycin-resistant enterococci (VRE) was first made in Europe and the United States in the late 1980's.[2,3] Since then, the incidence of resistance has increased. As a result, the ability to promptly detect and report VRE has become a concern of many clinicians.

Hardy Diagnostics HardyCHROM™ VRE is a differential and selective medium that allows for the rapid isolation of VRE from heavily contaminated specimens. HardyCHROM™ VRE uses chromogenic reactions to differentiate between *E. faecalis* and *E. faecium*, while traditional media, such as Bile Esculin Azide (BEA) agar with vancomycin, cannot differentiate between these two species. Differentiation is important because *E. faecium* is responsible for most VRE infections and has a higher vancomycin minimum inhibitory concentration (MIC) range than *E. faecalis*.[4]

There are many genes that code for vancomycin resistance, including *van*A, *van*B, *van*C, *van*D, and *van*E, and resistance is either intrinsic or acquired. *E. casseliflavus* and *E. gallinarum* have low intrinsic vancomycin resistance, while *E. faecalis* and *E. faecium* have moderate to high acquired resistance. Acquired resistance has also been found in other enterococcal species, such as *E. raffinosus*, *E. avium*, and *E. durans*.[5]

The CLSI (formerly NCCLS) breakpoint for vancomycin resistance is 32mcg/ml with intermediate susceptibility from 8 to 16mcg/ml. Most nosocomial outbreaks have been reported with MIC values well above 32mcg/ml. *E. faecalis* and *E. faecium* comprise more than 95% of resistant clinical isolates. *E. casseliflavus* and *E. gallinarum* are rarely isolated. With 10mcg/ml of vancomycin, HardyCHROM™ VRE will not support the growth of *E. casseliflavus* and *E. gallinarum,* owning to their low-level resistance.[6,7] Clinical relevance of the low-level VRE still is in question.[1]

The basal medium contains peptones to supply the necessary nutrients and chromogens to differentiate between *E. faecalis* and *E. faecium.* Selective agents are included to inhibit yeast, gram-negative bacteria, and non-*Enterococcus* gram-positive bacteria.

HardyCHROM™ VRE is available in a monoplate for patient specimen screening and a contact plate for environmental monitoring.

FORMULA

Ingredients per liter of deionized water:*

Peptones	20.0gm
Sodium Chloride	5.0gm
Chromogenic Mixture	5.0gm
Bile Salts	7.5gm
Selective Agents	1.0gm
Agar	15.0gm

Final pH 6.9 +/- 0.2 at 25 degrees C.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8 degrees C. 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. Chromogens are especially light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date applies to the product in its intact packaging when stored as directed.

This product has the following shelf life from the date of manufacture:

60 Days:	G333	HardyCHROM™ VRE
	P19	HardyCHROM™ VRE, Contact Plate

Refer to the keyword "Storage", in the Hardy Diagnostics' software program HUGO™, for more information on storing culture media.

PRECAUTIONS

This product is for *in vitro* diagnostic use only and 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". The "Guideline for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.

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 M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline.*

Sterilize all biohazard waste before disposal.

Refer to the keyword "Precautions", in the Hardy Diagnostics' software program HUGO™, for more information regarding general precautions when using culture media.

Refer to the keyword "MSDS", in the Hardy Diagnostics' software program HUGO™, for more information on handling potentially hazardous material.

PROCEDURE

Specimen Collection: Consult listed references for information on specimen collection.[9-11] Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport media and refrigerated until inoculation.

Consult the listed references for information regarding the processing of specimens.[8-12]

Protect media from light during storage and incubation as the product is light sensitive.

Method of Use - Agar Plates: Allow plates to warm to room temperature. The agar surface should be dry before inoculating. Inoculate and streak the specimen as soon as possible after collection. If the specimen to be cultured is on a swab, roll the swab over a small area of the agar surface. Streak for isolation with a sterile loop. Incubate plates aerobically at 35-37 degrees C. for 18-24 hours. Protect from light. Examine plates for colonies showing typical morphology and color after 24 hours. If no colonies are seen, reincubate for a total of 48 hours.

Do not incubate in an atmosphere supplemented with CO_2.

Method of Use - Contact Plates: Hold the plate with thumb and second finger and use index finger to press plate bottom firmly against the selected test surface. The same amount of pressure should be applied for every sample. Do not twist or move the plate laterally. Lateral movement spreads contaminants over the agar surface, thus making resolution of colonies difficult. A rolling motion may be used for slightly curved surfaces.[11]

Section or grid areas (walls, floors, etc.) to be assayed. Samples can then be taken from specific points within the grid.

Incubate plates aerobically at 35-37 degrees C. for 18-24 hours. Protect from light. Examine plates for colonies showing typical morphology and color after 24 hours. If no colonies are seen, reincubate for a total of 48 hours.

Do not incubate in an atmosphere supplemented with CO_2.

Using adequate light and magnification, count the number of colonies within the squares of the grid area. Take care not to count a square more than once. Using a Bactronic or Quebec colony counter, count colonies and record as the number of colonies per contact plate or number of colonies per square centimeter.[13]

Data should be collected and recorded according to a designed monitoring system that statistically provides for the accurate acquisition of data for multiple samples over time.

INTERPRETATION OF RESULTS

After incubation, the plates should show isolated colonies to facilitate identification. Isolated colonies are necessary for demonstration of typical color and morphology.

E. faecalis produces colonies that are dark red in color, and may have a golden sheen.

E. faecium produces colonies that are dark blue in color.

E. casseliflavus and *E. gallinarum* are inhibited.

Organism	Description	Photo	Color
E. faecalis	dark red colonies, with or without a golden sheen		
E. faecium	dark blue colonies		

LIMITATIONS

This medium is intended to be used as a screening medium. Further susceptibility testing of isolates should be performed for vancomycin following CLSI document M100: *Performance Standards for Antimicrobial Susceptibility Testing.*

It is recommended that further biochemical and/or serological tests be performed on colonies from pure culture for complete identification.

Using colonies isolated on HardyCHROM™ VRE to test for PYR activity may yield invalid results; PYR testing should be performed from a non-selective media.

Color-blind individuals may encounter difficulty in distinguishing the color differences on HardyCHROM™ VRE.

Minimize exposure of HardyCHROM™ VRE medium to light before and during incubation, as light can destroy the chromogens.

Low-level vancomycin-resistant strains of enterococci (e.g. *E. casseliflavus* and *E. gallinarum*) will not be detected on this medium. However, the clinical significance of low-level VRE still is in question.

Refer to the keyword "Limitations", in the Hardy Diagnostics' software program HUGO™, for more information regarding general limitations on culture media.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

The following organisms 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; dark red colonies
Enterococcus faecium ATCC® 700221	A	24hr	35°C	Aerobic	Growth; dark blue colonies
Enterococcus faecalis ATCC® 29212**	B	24hr	35°C	Aerobic	Inhibited
Enterococcus faecium ATCC® 9562	B	24hr	35°C	Aerobic	Inhibited
Enterococcus casseliflavus ATCC® 25788	B	24hr	35°C	Aerobic	Inhibited

* Refer to the keyword "Inoculation Procedures", in the Hardy Diagnostics' software program HUGO™, for a description of inoculation procedures.

** Recommended QC strains for User Quality Control according to the CLSI document M22 when applicable.

USER QUALITY CONTROL

Check for signs of contamination and deterioration. Users of commercially prepared media may be required to perform quality control testing with at least one known organism to demonstrate growth or a positive reaction; and at least one organism to demonstrate inhibition or a negative reaction (where applicable). Refer to the following keywords, in the Hardy Diagnostics' software program HUGO™, for more information on QC: "Introduction to QC", "QC of Finished Product", and "The CLSI (NCCLS) Standard and Recommendations for User QC of Media". Also see listed references for more information.[8-12]

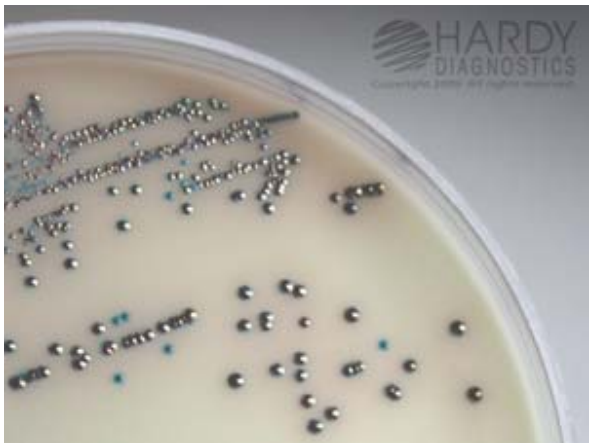
PHYSICAL APPEARANCE

HardyCHROM™ VRE should appear opaque, and cream to off-white with a green hue in color. May have a precipitate.

Vancomycin-resistant *Enterococcus faecalis* (ATCC® 51299) colonies growing on HardyCHROM™ VRE (Cat. no. G333). Incubated aerobically for 24 hours at 35 deg. C.

Vancomycin-resistant *Enterococcus faecium* (ATCC® 700221) colonies growing on HardyCHROM™ VRE (Cat. no. G333). Incubated aerobically for 24 hours at 35 deg. C.

Mixed culture of vancomycin-resistant *E. faecalis* and *E. faecium* colonies growing on HardyCHROM™ VRE (Cat. no. G333). Incubated aerobically for 24 hours at 35 deg. C.



Mixed culture of vancomycin-resistant *E. faecalis* and *E. faecium* colonies growing on HardyCHROM™ VRE (Cat. no. G333). Incubated aerobically for 48 hours at 35 deg. C.

Uninoculated plate of HardyCHROM™ VRE (Cat. no. G333).

REFERENCES

1. Jensen, Bette J. 1996. *Laboratory Medicine*; Vol. 27, No. 1, p. 53-55.

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

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

4. Fraser, S.L., et. al. 2009. *Enterococcal Infections*. eMedicine. www.emedicine.medscape.com/article/216993-overview.

5. Centers for Disease Control and Prevention. 2010. *Vancomycin-resistant Enterococci (VRE) and the Clinical Laboratory*. http://cdc.gov/HAI/settings/lab/VREClinical-Laboratory.html.

6. Verbal communication with Janet Hindler. 1997. UCLA Medical Center, July.

7. *Clinical-Microbiology Newsletter*. 1998. Vol. 20, No. 1. Elsevier Science, Inc., New York, New York.

8. Anderson, N.L., et al. 2005. *Cumitech 3B; Quality Control and Quality Assurance Practices in Clinical Microbiology*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

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

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

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

12. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

13. U.S. Food and Drug Administration. *Bacteriological Analytical Manual.* AOAC, Arlington, VA. www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm.

ATCC is a registered trademark of the American Type Culture Collection.

031611db

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

Distribution Centers:
California · Washington · Utah · Arizona · Texas · Ohio · Florida

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

KEY diagnostics
Tel : 02 8212 4074
Fax: 02 9423 6692
www.keydiagnostics.com.au
PO Box 1038 Gymea NSW 2227