IFU

Instructions for Use

CRITERION™ HARDYCHROM™ VIBRIO

Cat. no. C9010	CRITERION™ HardyCHROM™ Vibrio	160.5g
Cat. no. C9011	CRITERION™ HardyCHROM™ Vibrio	500g
Cat. no. C9012	CRITERION™ HardyCHROM™ Vibrio	2kg
Cat. no. C9013	CRITERION™ HardyCHROM™ Vibrio	10kg

INTENDED USE

Hardy Diagnostics CRITERION™ HardyCHROM™ Vibrio is recommended for use as a selective and differential growth medium for the cultivation, isolation, and differentiation of *Vibrio* spp. from food and environmental samples.

This dehydrated culture medium is a raw material intended to be used in the making of prepared media products, which will require further processing, additional ingredients, or supplements.

SUMMARY AND PRINCIPLES

The *Vibrio* genus is comprised of Gram-negative, halophilic, non-spore forming rods that are straight or have a single, rigid curve. All *Vibrio* spp. are motile, and most species are oxidase- and catalase-positive. Several species in the genus are known human pathogens, the most notable of which are *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus*.[1-5] Most disease-causing strains are associated with gastroenteritis following ingestion of raw or uncooked shellfish or drinking contaminated water. Cases of open wound infection and septicemia have also been reported.[4,6]

V. cholerae is the most well known *Vibrio* species, as it is the causative agent of cholera. This organism is most often transmitted via contaminated drinking water in developing countries.[1,7] *V. parahaemolyticus* is the leading cause of bacterial diarrhea associated with seafood consumption. It is transmitted through ingestion of undercooked or mishandled seafood, or less commonly through open wounds exposed to seawater.[5,8] While not as common, *V. vulnificus* is the most fatal *Vibrio* species in the United States. Infection and death from this species occurs from consumption of Gulf Coast oysters and possibly wound infections acquired in or exposed to marine environments.[4,9]

CRITERION™ HardyCHROM™ Vibrio Agar is a chromogenic medium, highly selective for isolation of *V. parahaemolyticus*, *V. vulnificus*, *V. cholerae* and *V. alginolyticus*, as well as other *Vibrio* species. HardyCHROM™ Vibrio Agar has a high pH, which suppresses the growth of non-*Vibrio* species found in similar marine samples.[10] The media consists of animal proteins, sea salts, sodium citrate, sodium thiosulfate, sugars, and a chromogenic mix. Seas salts are incorporated into the medium to provide optimum growth and metabolic activity of halophilic *Vibrio* spp. Sodium thiosulfate provides a source of sulfur. The inclusion of chromogenic substrates allows for the differentiation of *Vibrio* species based on colony color. It is the only medium that will differentiate *V. vulnificus* from *V. parahaemolyticus* and *V. cholerae*.

FORMULA

Ingredients per liter of deionized water:*

Gram weight per liter:	80.25g/L
Peptone	10.0g
Sea Salt Mixture	10.0g
Oxbile	10.0g
Sodium Thiosulfate	10.0g
Sucrose	10.0g
Yeast Extract	5.0g
Sodium Citrate	5.0g
Sodium Carbonate	1.5g
Lactose	2.0g
Sodium Pyruvate	0.5g
Chromogenic Mixture	1.25g
Agar	15.0g

Final pH 8.6 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Store the sealed bottle(s) containing dehydrated culture medium at 2-30ºC. Dehydrated culture medium is very hygroscopic. Keep lid tightly sealed. Protect dehydrated culture media from moisture and light. The dehydrated culture media should be discarded if it is not free-flowing or if the color has changed from its original white to off-white.

Store the prepared culture media at 2-8ºC. Prepared culture media is light and temperature sensitive; protect from light, excessive heat, moisture, 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 blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory 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.

METHOD OF PREPARATION FOR DEHYDRATED CULTURE MEDIA

1. Suspend 80.25g of the dehydrated culture medium in one liter of distilled or deionized water. Stir to mix thoroughly.

2. Heat to boiling and mix to dissolve completely. Do not overheat.

3. **Do not autoclave.**

4. Cool to 45-50ºC and dispense as desired.

NOTE: If enhanced differentiation of *V. parahaemolyticus* and *V. alginolyticus* is required, 1.25ml/L of a sterile 1% TTC solution (Cat. no. 231121) should be aseptically added to the medium at step 4 and mixed thoroughly just prior to dispensing.

PROCEDURE

Sample collection preparation: Infectious material should be submitted directly to the laboratory within two to three hours of collection. Samples should be protected from excessive heat and cold. If there will be a delay in processing, the sample should be inoculated onto an appropriate transport medium and refrigerated until inoculation. Consult listed references for information on sample collection and preparation.[1-6]

Method of use: Plates should be warmed to room temperature and the agar surface should be dry prior to inoculating. Chapter Nine of the FDA Bacteriological Analytical Manual (BAM) has specific recommendations for the optimal recovery of *Vibrio* from food and water specimens.[6] When dilutions are necessary, Phosphate Buffered Saline (Cat. nos. K146, K161, or K163) serves as an appropriate diluent. Specimen and sample dilutions may be placed into Alkaline Peptone Water (Cat. no. K03) for 16-24 hours at 35ºC as a generic enrichment for *Vibrio*, but the optimal enrichment broth, temperature and incubation time will vary depending upon the specimen or sample matrix and the target organism(s). Inoculate a 3mm loopful of enrichment broth onto HardyCHROM™ Vibrio and streak for isolation. Incubate aerobically at 35ºC for 18-24 hours and examine for characteristic colonial morphology. If after 24 hours *Vibrio* spp. are not detected, plates should be discarded.

INTERPRETATION OF RESULTS

Examine plates for isolated colonies showing typical morphology and color.

Vibrio parahaemolyticus produces colonies that are teal in color.

Vibrio cholerae produces colonies that are magenta to purple in color and do not fluoresce under UV light (365nm).

Vibrio vulnificus produces colonies that are magenta to purple in color and do fluoresce under UV light (365nm).

Vibrio alginolyticus produce colonies that range from clear with teal centers to completely teal. **NOTE:** When TTC is added to the medium, *V. alginolyticus* and other *Vibrio* spp. will produce olive/brown colonies.

Other, less common *Vibrio* spp. may produce colonies that vary in color and can include clear, teal and brown.

Enterococcus faecalis may grow on HardyCHROM™ Vibrio, but colonies appear sky blue.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies

from pure culture for complete identification.

Some formulations may require a settling period before pH testing of the prepared medium. If the pH is tested immediately after preparation and is out of specification, retest the medium after 24 hours to obtain final pH results. Always take pH reading at room temperature.

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

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

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media or enrichment broths such as Phosphate Buffered Saline (Cat. nos. K146, K161, or K163) or Alkaline Peptone Water (Cat. no. K03), incinerators, and incubators, etc., as well as UV light and 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	
Vibrio parahaemolyticus ATCC® 17802	B	24hr	35°C	Aerobic	Growth; teal colonies
Vibrio vulnificus ATCC® 27562	B	24hr	35°C	Aerobic	Growth; magenta to purple colonies, fluorescence under UV
Vibrio cholerae ATCC® 9459	B	24hr	35°C	Aerobic	Growth; magenta to purple colonies, no fluorescence under UV
Vibrio alginolyticus ATCC® 17749	B	24hr	35°C	Aerobic	Growth; clear colonies with teal centers to completely teal colonies
Escherichia coli ATCC® 25922	B	24hr	35°C	Aerobic	Partial to complete inhibition

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

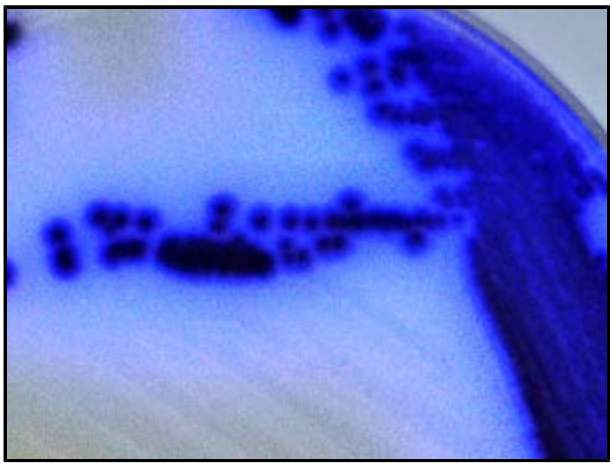
USER QUALITY CONTROL

Users of dehydrated 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. In addition, refer to the following document "Finished Product

Quality Control Procedures," for more information on QC or see the reference(s) for more specific information.

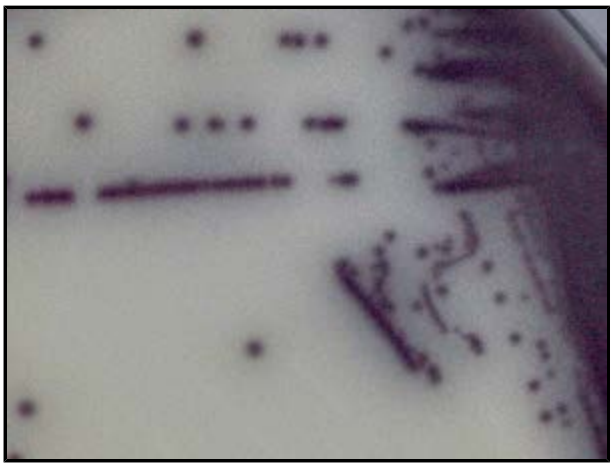
PHYSICAL APPEARANCE

CRITERION™ HardyCHROM™ Vibrio powder should appear homogeneous, free-flowing and white to off-white in color. The prepared medium should appear opaque and off-white in color.



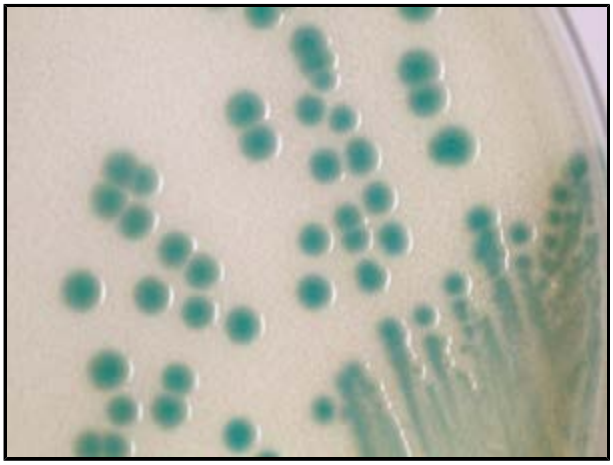
Vibrio vulnificus (ATCC® 27562) colonies growing on HardyCHROM™ Vibrio (Cat. no. G319) under UV. Incubated aerobically for 24 hours at 35ºC.

Vibrio vulnificus (ATCC® 27562) colonies growing on HardyCHROM™ Vibrio (Cat. no. G319). Incubated aerobically for 24 hours at 35ºC.



Vibrio cholerae (ATCC® 9459) colonies growing on HardyCHROM™ Vibrio (Cat. no. G319) under UV. Incubated aerobically for 24 hours at 35ºC.

Vibrio cholerae (ATCC® 9459) colonies growing on HardyCHROM™ Vibrio (Cat. no. G319). Incubated aerobically for 24 hours at 35ºC.



Vibrio parahaemolyticus (ATCC® 17802) colonies growing on HardyCHROM™ Vibrio (Cat. no. G319). Incubated aerobically for 24 hours at 35ºC.

Vibrio alginolyticus (ATCC® 17749) colonies growing on HardyCHROM™ Vibrio (Cat. no. G319). Incubated aerobically for 24 hours at 35ºC.

REFERENCES

1. Kaper, J. B., J. G. Morris Jr., and M. M. Levine. 1995. Cholera. *Clin.Microbiol. Rev.* 8(1):48-86.

2. McLaughlin, J.C 1995. Vibrio, p 465-476. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenevor,and R. H. Yolken (eds.), Manual of Clinical Microbiology, 6th ed. ASM Press, Washington, DC.

3. McPherson, V. L., J.A. Watts, L. M. Simpson, and J. D. Oliver. 1991. Physiological effects of the lipopolysaccharide of Vibrio vulnificus on mice and rats. *Microbios.* 67:141-149.

4. Oliver, J. D. 1989. Vibrio vulnificus, p 569-600. *In* M.P. Doyle (ed.), Foodborne Bacterial Pathogens. Marcel Dekker, Inc., New York, New York.

5. Sakazaki, R., S. Iwanami, and H. Fukumi.1963. Studies on the enteropathogenic, facultatively halophilic bacteria, *Vibrio parahaemolyticus*. I. Morphological, cultural and biochemical properties and its taxonomic position. Jpn. J. Med. Sci. Biol. 16:161-188.

6. Rippey, S. R. 1994. Infectious diseases associated with molluscan shellfish consumption. *Clin. Microbiol. Rev.* 7:419-425.

7. Sack, D. A, R. B Sack, G. B. Nair, and A. K. Siddique. 2004. Cholera. *Lancet.* 363:223-233.

8. Klonts, K. C., L. Williams, L. M. Baldy, and M. Campos.1993. Raw oyster-associated Vibrio infections: Linking epidemiologic data with laboratory testing of oysters obtained from a retail outlet. *J. Food Protect.* 56:977-979.

9. Tacket, C. O., F. Brenner, and P. A. Blake. 1984. Clinical features and an epidemiological study of Vibrio vulnificus infections. *J. Infect. Dis.* 149:558-561.

10. U.S. Food and Drug Administration. *Bacteriological Analytical Manual.* Arlington, VA. http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm

11. Ajello, et al. 1963. *CDC Laboratory Manual for Medical Mycology* , PHS Publication No. 994, U.S. Government Printing Office, Washington, D.C.

12. Versalovic, J., et al. *Manual of Clinical Microbiology.* American Society for Microbiology, Washington, D.C.

13. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology,* C.V. Mosby Company, St. Louis, MO.

IFU-10171[C]

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.