

Instructions for Use

HARDYCHROM™ VIBRIO

Cat. no. G319	HardyCHROM™ Vibrio, 15x100mm Plate, 18ml	10 plates/bag
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INTENDED USE

Hardy Diagnostics 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 product is not intended to be used for the diagnosis of human disease.

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)

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:*

Peptone	10.0g
Sea Salt Mixture	10.0g
Ox bile	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 25°C.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°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. Product 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.

PROCEDURE

Sample collection and 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. Sample dilutions may be placed into Alkaline Peptone Water (Cat. no. K03) for 16-24 hours at 35°C as a generic enrichment for *Vibrio*, but the optimal enrichment broth, temperature and incubation time will vary depending upon the sample matrix and the target organism(s). Inoculate a 3mm loopful of enrichment broth onto HardyCHROM™ *Vibrio* and streak for isolation. Incubate aerobically at 35°C 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.

Use a 365nm wavelength handheld UV Lamp ([Cat. no. UVL56](#) or [LSS3](#)) to detect colony fluorescence. These handheld lamps require that the room lights be turned off, since ambient light will interfere with fluorescence detection. Alternatively, a dark viewing box ([Cat. no. CM10A](#)) with its companion UV lamp ([Cat. no. EA160](#)) may be used so that the room lights will not need to be turned off.

CAUTION: Not all UV wavelengths are capable of producing sufficient fluorescence effects. It is important to use a UV light with a wavelength at or near 365nm, one with higher power (in watts, not lumens), and one that is high efficiency. Use of UV lights not meeting these criteria will fail to produce sufficient fluorescence. Most inexpensive battery operated LED UV lights produce light at multiple wavelengths, use less watts, and/or low power, and are thus **not acceptable** and will produce erroneous results. [Cat. no. LSS3](#) is an exception and has been verified to work well. Please do not use cheaper versions.

Tips for using fluorescence

1. Use a 365nm handheld UV lamp ([Cat. no. UVL56](#)) or ([Cat. no. LSS3](#)) to detect colony fluorescence. See 'CAUTION' above regarding inexpensive handheld UV lights. Alternatively, a dark viewing box with its compatible UV lamp may be used as described above. Viewing must be done in the dark.
2. Hold the lamp directly over isolated colonies on the plate, approximately 3 to 4 inches (7 to 10cm) away.
3. Isolated colonies of *Vibrio vulnificus* and *Vibrio parahaemolyticus* will fluoresce a blue glow.
4. Only well isolated colonies will fluoresce. Colonies in areas of confluent growth will not.
5. Fluorescence will fade over time, as colonies develop to a darker pigment.

Interpretation

Vibrio parahaemolyticus produces colonies that are torquoise in color, and fluoresce blue.

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 in color and will fluoresce blue under UV light (365nm).

Vibrio alginolyticus and other *Vibrio* spp. produce colonies that are colorless to olive in color and do not fluoresce.

Enterococcus faecalis may grow on HardyCHROM™ *Vibrio*, but colonies appear sky blue and do not fluoresce.

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.

Fluorescence must be read in a darkened environment with a 365nm wavelength UV lamp of adequate power (see “Tips for Using Fluorescence” above).

Color-blind individuals may encounter difficulty in distinguishing 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. no. K146](#) or [Cat. no. K163](#)) or Alkaline Peptone Water ([Cat. no. K03](#)), incinerators, and incubators, etc., as well as handheld UV lamp ([Cat. no. UVL56](#) or [LSS3](#)) or dark viewing box ([Cat. no. CM10A](#)) with compatible UV lamp ([Cat. no. EA160](#)), 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	
<i>Vibrio parahaemolyticus</i> ATCC® 17802	B	24hr	35°C	Aerobic	Growth; torquoise colonies, fluoresce blue
<i>Vibrio vulnificus</i> ATCC® 27562	B	24hr	35°C	Aerobic	Growth; magenta colonies, fluoresces blue under UV light
<i>Vibrio cholerae</i> ATCC® 9459	B	24hr	35°C	Aerobic	Growth; magenta to purple colonies, no fluorescence under UV light
<i>Vibrio alginolyticus</i> ATCC® 17749	B	24hr	35°C	Aerobic	Growth; colorless to olive colonies, no fluorescence
<i>Escherichia coli</i> ATCC® 25922	B	24hr	35°C	Aerobic	Partial to complete inhibition

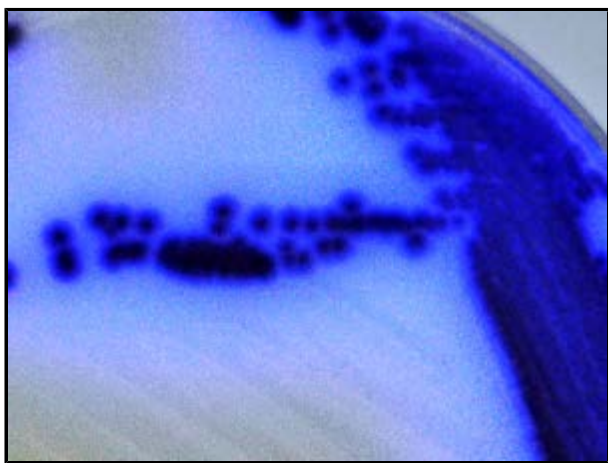
* Refer to the document "[Inoculation Procedures for Media QC](#)" for more information.

USER QUALITY CONTROL

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

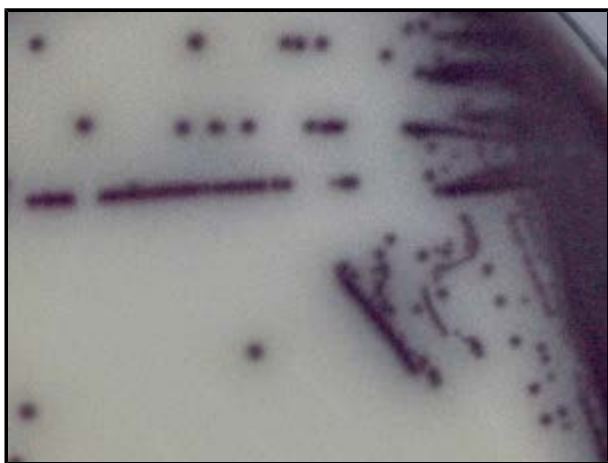
HardyCHROM™ Vibrio should appear opaque and off-white in color.



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



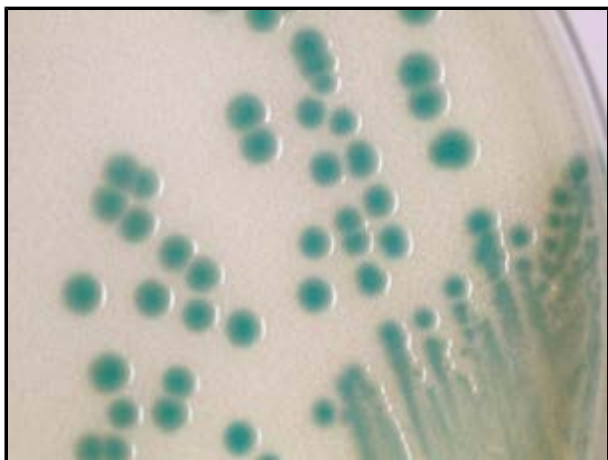
Vibrio vulnificus (ATCC® 27562) colonies growing on HardyCHROM™ Vibrio (Cat. no. G319). Incubated aerobically for 24 hours at 35°C.



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



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



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



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

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