67631 Vibrio Millichrome™ plus Agar

For isolation and detection of *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae*.

Composition:

Ingredients	Grams/Litre
Peptone and yeast extract	8.0
Salts	51.4
Chromogenic mix*	0.3
Agar	15.0

Final pH 9.0 +/- 0.2 at 25°C

* confidential mix with chromogenic substrates

Store prepared media at 2-8°C, protected from direct light and dehydration (max. 1 month) or 1 day at room temperature. Store dehydrated powder, in a dry place, in tightly sealed containers at 2-25°C.

Preparation:

Step 1 (Preparation of 1L media)

- Disperse slowly 74.7 g of powder base in 1 L of purified water.
- Stir until agar is well thickened.
- Heat and bring to boil (100 °C) while swirling or stirring regularly.

DO NOT HEAT TO MORE THAN 100 °C. DO NOT AUTOCLAVE AT 121 °C.

Warning 1: If using an autoclave, do so without pressure.

Advice 1: For the 100 °C heating step, mixture may also be brought to a boil in a microwave oven: after initial boiling, remove from oven, stir gently, then return to oven for short repeated bursts of heating until complete fusion of the agar grains has taken place (large bubbles replacing foam).

Step 2 (Pouring)

- Cool in a water bath at 45-50 °C, swirling or stirring gently.

Advice 2: In case of samples with a high presence of Aeromonas, 50 mg of cefsulodin can be added to the mix once cooled down at 45-50 °C (50 mg/L).

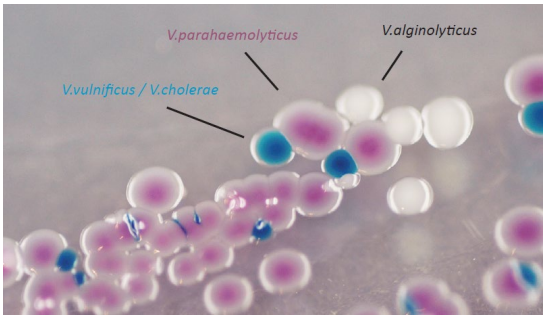
- Pour medium into sterile Petri dishes.
- Let it dry and gel

Principle and Interpretation:

The natural reservoir of *Vibrio* are marine plants and animals, but only four of 20 species are linked to serious diseases.

V*. cholerae* often cause cholera through water and food contamination. Emerging cyclically, cholera is considered to be endemic in many countries as a virulent disease causing severe diarrhea and dehydration. *V. parahaemolyticus* and *V. vulnificus* are largely involved in foodborne diseases from seafood, causing septicaemia, wound infections, and gastroenteritis. Despite the fact that *V. parahaemolyticus* is the most commonly reported species causing infection, *V. vunificus* has become increasingly prevalent and is now associated with 94 % of reported deaths. *V. alginolyticus* is less common but is a pathogen concern for oyster producers since it can lead to major production losses. If detected, it can prevent contamination of other oyster production sites. Compared to TCBS (based on

detection of sucrose fermentation with a pH indicator) this medium has clear color differentiation *V. parahaemolyticus* grows as mauve colonies while *V. cholerae and V. vulnificus give blue colonies*. *V. alginolyticus* remains colorless which helps to avoid any interference with the detection of other species. The recovery rate of Vibrio Millichrome™ plus Agar is higher than with TCBS agar, even if using an enrichment broth. Also, fewer false negatives results are obtained than with TCBS agar.



Peptones and yeast extract provide nitrogenous nutrients for growth and other essential growth factors. Salts are needed for the osmotic balance, stimulates the growth, provide essential ions and in this high concentration it gives together with the alkaline pH selectivity to Vibrio species. The chromogenic mix contains chromogenic substrates for the color differentiation based on the ability to cleave the substrate by characteristic enzymes. Agar is added as the solidifying agent.

Limitation and further testing

- The final identification must be confirmed by biochemical tests or by mass spectrophotometry (eg. MALDI-TOF), use suspicious colonies.
- For the Oxidase test of blue colonies, we suggest the use of normal Oxidase test.
- For the Oxidase test of mauve colonies, we suggest the use of a reagent giving a blue colour with oxidase positive bacteria (tetramethyl-p-phenylenediamine solution at 10 mg/mL).

Quality control:
Cultural characteristics after 24h at 37°C.

Organisms (ATCC/WDCM)	Growth	Colony color
Vibrio vulnificus (27562/00139)	+++	green
Vibrio parahaemolyticus (33845/-)	+++	mauve
Vibrio alginolyticus (33839/-)	+++	creamy
Staphylococcus aureus (25923/00034)	-	-
Escherichia coli (25922/00013)	-	-

References:

1. Suriya Palamae et al., Vibrio parahaemolyticus Isolates from Asian Green Mussel: Molecular Characteristics, Virulence and Their Inhibition by Chitooligosaccharide-Tea Polyphenol Conjugates, Foods (2022)
2. N.W. Hammood, I.A.J. Ibrahim, Bacterial Content in Gut for different Species of Fish Collecting from Tigris River in Baghdad City, Iraq, Tikrit Journal of Pure Science (2018)
3. Tran Thi Hong To et al., Prevalence of Vibrio parahaemolyticus Causing Acute Hepatopancreatic Necrosis Disease of Shrimp in Shrimp, Molluscan Shellfish and Water Samples in the Mekong Delta, Vietnam, Biology, (2020)

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.