



Ready Prepared Media

Technical Information

TCBS Agar Plate

Product Code: PM 1870

Application: Recommended for the selective isolation of *Vibrio cholerae* and other enteropathogenic *Vibrio*'s

Composition**

Ingredients	Gms / Litre
Peptone, special	10.000
Yeast extract	5.000
Sodium citrate	10.000
Sodium thiosulphate	10.000
Sodium cholate	3.000
Bile#	5.000
Sucrose	20.000
Sodium chloride	10.000
Ferric citrate	1.000
Bromo thymol blue	0.040
Thymol blue	0.040
Agar	15.000
Final pH (at 25°C)	8.8±0.2

**Formula adjusted, standardized to suit performance parameters# Equivalent to Oxgall

Principle & Interpretation

TCBS Agar was developed by Kobayashi et al (1), who modified the selective medium of Nakanishi (2). Although this medium was originally designed for the isolation of *V. cholerae* and *V. parahaemolyticus*, most *Vibrios* grow to healthy large colonies with many different colonial morphologies. TCBS Agar is also recommended by APHA for the selective isolation of *V. cholerae* and *V. parahaemolyticus* (1,3). Enrichment in Alkaline Peptone Water (M618), followed by isolation on TCBS Agar is routinely used for isolation of *V. cholerae* (2,4,5). TCBS Agar, Selective has an additional selective ingredient i.e. sodium cholate for improved selectivity.

Peptone special and yeast extract provide nitrogenous, carbonaceous compounds, long chain amino acids, vitamin B complex and other essential growth nutrients. Bile and sodium citrate inhibit gram-positive bacteria and coliforms (6). Sodium thiosulphate serves as a good source of sulphur, which in combination with ferric citrate detects the production of hydrogen sulphide. For the metabolism of *Vibrios*, sucrose is added as a fermentable carbohydrate. *Vibrio* that is able to utilize sucrose will form yellow colonies. Bromothymol blue and thymol blue are the pH indicators. The alkaline pH of the medium improves the recovery of *V. cholerae*. Strains of *V. cholerae* produce yellow colonies on TCBS Agar because of fermentation of sucrose. *V. alginolyticus* also produce yellow colonies. *V. parahaemolyticus* is a sucrose non-fermenting organism and therefore produces blue-green colonies, as does *V. vulnificus*. *Proteus* species that are sucrose-fermenters may form yellow colonies (7). TCBS Agar is not a suitable medium for oxidase testing of *Vibrio* species (8). A few strains of *V. cholerae* may appear green or colourless on TCBS Agar due to delayed sucrose fermentation (7). TCBS Agar is highly selective for *Vibrio* species. However, occasional isolates of *Pseudomonas* and *Aeromonas* may also form blue green colonies on TCBS Agar (8). Any H₂S negative colony of TCBS Agar can be considered presumptive positive for *Vibrio*.



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Type of specimen

Clinical : faeces, etc; Food samples; Water samples

Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (4). For food samples, follow appropriate techniques for sample collection and processing as per guidelines (6). For clinical samples follow appropriate techniques for handling specimens as per established guidelines (9,10). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use only. For professional use only. Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

- (1) The medium should be inoculated heavily with faecal specimens because growth of few species may be inhibited on the medium due to fermentation of sucrose and accumulation of acids.
- (2) However, occasional isolates of *Pseudomonas* and *Aeromonas* may also form blue green colonies on TCBS Agar (8).
- (3) *Proteus* species that are sucrose-fermenters may form yellow colonies (8).
- (4) TCBS Agar is not a suitable medium for oxidase testing of *Vibrio* species (9).
- (5) A few strains of *V.cholerae* may appear green or colourless on TCBS Agar due to delayed sucrose fermentation.
- (6) TCBS Agar is highly selective for *Vibrio* species. Any H₂S negative colony of TCBS Agar can be considered presumptive positive for *Vibrio*.
- (7) Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
- (8) Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
- (9) Further biochemical and serological tests must be carried out for complete identification.
- (10) It is recommended to store the plates at 24-30°C to avoid minimum condensation

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Methodology

Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate.

Quality Control

Appearance

Sterile TCBS Agar in 90 mm disposable Petri plates with smooth surface and absence of black particles/cracks/bubbles

Colour of medium

Bluish green coloured medium



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Quantity of medium

25 ml of medium in 90 mm disposable plates.

Reaction

8.60-9.00

Sterility Test

Passes release criteria

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours .

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Vibrio parahaemolyticus ATCC 17802 (00037*)	50-100	good-luxuriant	>=50%	bluish green
Vibrio vulnificus ATCC 29306	50-100	fair-good	>=30%	greenish yellow
Vibrio fluvialis ATCC33809 (00137*)	50-100	good-luxuriant	>=50%	yellow
Vibrio cholerae ATCC 15748	50-100	good-luxuriant	>=50%	yellow
Escherichia coli ATCC25922 (00013*)	>=10 ³	inhibited	0%	
Enterococcus faecalis ATCC29212 (00087*)	>=10 ³	inhibited	0%	
Shigella flexneri ATCC12022 (00126*)	>=10 ³	inhibited	0%	

Key : * - Corresponding WDCM numbers

Storage and Shelf Life

On receipt store between 20-30°C. Use before expiry date on the label. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (11,12).

Further Reading

1. Kobayashi T., Enomoto S., Sakazaki R., and Kuwahara S., 1963, Jap. J. Bacteriol., 18: 387.
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5. Morris G. K., Merson M. H., Huq A. K., Kibria A. K. and Black R., 1979, J. Clin. Microbiol., 9:79.
6. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
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8. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams & Wilkins, Baltimore, Md.
9. Forbes B. A., Sahm A. S. and Weissfeld D. F., 1998, Bailey & Scotts Diagnostic Microbiology, 10th Ed., Mosby, Inc. St. Louis, Mo.
10. Furniss A. L., Lee J. V. and Donovan T. J., 1978, The Vibrios, Public Health Laboratory Service Monograph Series No. 11, Maidstone Public Health Laboratory, H.M.S.O., London, England.
11. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
12. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock, D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.



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Disclaimer

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