Technical Information

TCBS Agar

Product Code: DM 1189

Application: TCBS Agar is recommended for the selective isolation and cultivation of *Vibrio cholerae* and other enteropathogenic *Vibrio's* causing food poisoning.

Composition**

Ingredients	Gms / Litre
Proteose peptone	10.000
Yeast extract	5.000
Sodium thiosulphate	10.000
Sodium citrate	10.000
Oxgall	8.000
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)	8.6±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

TCBS Agar was developed by Kobayashi et al [1]. He modified the selective medium of Nakanishi [2]. Although this medium was originally devised for the isolation of *V.cholerae* and *V. parahaemolyticus,* most *Vibrios* grew with different colonial morphologies. TCBS Agar is also recommended by APHA for the selective isolation of *V. cholerae* and *V.parahaemolyticus* [3,4]. Enrichment in Alkaline Peptone Water (DM618), followed by isolation on TCBS Agar is routinely used for isolation of *V.cholerae* [5-7] from different clinical sampls.

Proteose peptone and yeast extract provide nitrogenous compounds, vitamin B complex and other essential growth nutrients. Oxgall, a derivative of bile salts and sodium citrate inhibit the growth a gram-positive bacteria and coliforms [8]. 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 from 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 [9]. However, occasional isolates of *Pseudomonas* and *Aeromonas* may also form blue green colonies on TCBS Agar [9]. Any H_2S negative colony of TCBS Agar can be considered presumptive positive for *Vibrio.* TCBS Agar is not a suitable medium for oxidase testing of *Vibrio* species [10]. A few strains of *V. cholerae* may appear green or colourless on TCBS Agar due to delayed sucrose fermentation [9].

Methodology

Suspend 89.08 grams of powder media in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 50°C and pour into sterile Petri plates.

Quality Control

Physical Appearance
Light yellow to light tan homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Bluish green coloured clear to slightly opalescent gel forms in Petri plates.

Reaction
Reaction of 8.9% w/v aqueous solution at 25°C. pH : 8.6±0.2

pH Range:- 8.40-8.80

Cultural Response/Characteristics
DM1189: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum(CFU)	Growth	Recovery	Colour ofcolony
Enterococcus faecalis ATCC 29212	>=10^3	inhibited	0%	
Escherichia coli ATCC 25922	>=10^3	inhibited	0%	
Shigella flexneri ATCC 12022	>=10^3	inhibited	0%	
Vibrio cholerae ATCC 15748	50-100	good-luxuriant	>=50%	yellow
Vibrio fluvialis ATCC 33809	50-100	good-luxuriant	>=50%	yellow
Vibrio parahaemolyticus ATCC 17802	50-100	good-luxuriant	>=50%	bluish green
Vibrio vulnificus ATCC 29306	50-100	fair-good	>=30%	greenish yellow

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

1. Kobayashi T., Enomoto S., Sakazaki R., and Kuwahara S., 1963, Jap. J. Bacteriol., 18: 387.
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5. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Eds.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
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8. Howard B., 1994, Clinical and Pathogenic Microbiology, 2nd Ed., The C.V. Mosby.
9. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams & Wilkins, Baltimore, Md.
10. Morris G. K., Merson M. H., Huq A. K., Kibrya A. K. and Black R., 1979, J. Clin. Microbiol., 9:79

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