TCBS Agar

M189

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

Directions

Suspend 89.08 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 50°C and pour into sterile Petri plates.

Principle And 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* (3,4). Enrichment in Alkaline Peptone Water (M618), followed by isolation on TCBS Agar is routinely used for isolation of *V.cholerae* (5-7).

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 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). 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).

TCBS Agar is highly selective for *Vibrio* species. However, occasional isolates of *Pseudomonas* and *Aeromonas* may also form blue green colonies on TCBS Agar (9). Any H2S negative colony of TCBS Agar can be considered presumptive positive for *Vibrio* .

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.

Quality Control

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
8.40-8.80

Cultural Response
M189: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Cultural Response				
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
Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference
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