TCBS Agar

M189

Intended Use:

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

Composition**

Ingredients	Gms / Litre
Proteose peptone	10.000
Yeast extract	5.000
Sodium thiosulphate	10.000
Sodium citrate	10.000
Bile	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 45-50°C. Mix well 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. Bile, 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*.

Type of specimen

Clinical : faeces ; Food samples; Water samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,12).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,11,13)

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(4)

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic 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 (9).
3.*Proteus* species that are sucrose-fermenters may form yellow colonies (9).
4.TCBS Agar is not a suitable medium for oxidase testing of Vibrio species (10).
5.A few strains of V. cholerae may appear green or colourless on TCBS Agar due to delayed sucrose fermentation (9).
6.TCBS Agar is highly selective for *Vibrio* species. Any H2S negative colony of TCBS Agar can be considered presumptive positive for Vibrio .
6. Further biochemical and serological tests must be carried out for complete identification

Performance and Evaluation

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

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

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Cultural Response				
Shigella flexneri ATCC 12022 (00126*)	>=10^3	inhibited	0%	
Vibrio cholerae ATCC 15748	50-100	good-luxuriant	>=50%	yellow
Vibrio fluvialis ATCC 33809 (00137*)	50-100	good-luxuriant	>=50%	yellow
Vibrio parahaemolyticus ATCC 17802 (00037*)		good-luxuriant	>=50%	bluish green
Vibrio vulnificus ATCC 29307 (00187*)	50-100	fair-good	>=30%	greenish yellow
Enterococcus faecalis ATCC 29212 (00087*)	>=10^3	inhibited	0%	
Escherichia coli ATCC 25922 (00013*)	>=10^3	inhibited	0%	
Proteus vulgaris ATCC 13315	>=10^3	inhibited	0%	

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store below 30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. 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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,12).

Reference

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11. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
12. Isenberg, H.D. Clinical Microbiology Procedures Handb0ook. 2nd Edition.
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IVD	In vitro diagnostic medical device
CE	CE Marking
30°C 10°C	Storage temperature
	Do not use if package is damaged
	HiMedia Laboratories Pvt. Limited, 23 Vadhani Industrial Estate, LBS Marg,Mumbai-86,MS,India
EC REP	CE Partner 4U ,Esdoornlaan 13, 3951 DB Maarn The Netherlands, www.cepartner 4u.eu

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