



TCBS Agar (Selective)

M870

Intended Use:

Recommended for 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

Directions

Suspend 89.08 grams in 1000 ml purified / 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). TCBS Agar, Selective has an additional selective ingredient i.e. sodium cholate for improved selectivity.

Peptone special and yeast extract provide nitrogenous compounds, vitamin B complex and other essential growth nutrients. Bile 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 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 (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.

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 (11,12).

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

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.
3. *Proteus* species that are sucrose-fermenters may form yellow colonies.
4. TCBS Agar is not a suitable medium for oxidase testing of *Vibrio* species.
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. Further biochemical and serological tests must be carried out for complete identification.

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.

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 : 8.8±0.2

pH

8.60-9.00

Cultural Response

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

Organism	Growth	Inoculum (CFU)	Recovery	Colour of colony
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<i>Escherichia coli</i> ATCC 25922 (00013*)	inhibited	$\geq 10^4$	0%	
<i>Vibrio parahaemolyticus</i> ATCC 17802 (00037*)	good-luxuriant	50-100	$\geq 50\%$	bluish green
<i>Vibrio vulnificus</i> ATCC 29306	fair-good	50-100	$\geq 30\%$	greenish yellow
<i>Vibrio fluvialis</i> ATCC 33809 (00137*)	good-luxuriant	50-100	$\geq 50\%$	yellow
<i>Enterococcus faecalis</i> ATCC 29212 (00087*)	inhibited	$\geq 10^4$	0%	
<i>Vibrio cholerae</i> ATCC 15748	good-luxuriant	50-100	$\geq 50\%$	yellow
<i>Shigella flexneri</i> ATCC 12022 (00126*)	inhibited	$\geq 10^4$	0%	

Key: (*) Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°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

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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 (11,12).

Reference

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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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