



TCBS Agar, Modified

M870A

Intended Use:

Recommended for selective isolation of *Vibrio cholerae* and other enteropathogenic *Vibrio*'s from clinical and non-clinical samples.

Composition**

Ingredients	g/ L
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	14.000
Final pH (at 25°C)	8.6±0.1

**Formula adjusted, standardized to suit performance parameters

Equivalent to Oxgall

Directions

Suspend 88.08 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. Dry the plates overnight or at 37-45°C before use.

Principle And Interpretation

TCBS Agar was first formulated by Nakanishi (1) and further modified by Kobayashi et al (2). It promotes rapid growth of pathogenic *Vibrio*'s after 24 hours incubation at 37°C. The contaminating non-*vibrio*'s are suppressed. Peptone special and yeast extract provide nitrogenous and carbonaceous compounds, long chain amino acids, vitamin B complex and other essential growth nutrients. Bile a derivative of bile salts and the sodium citrate inhibit gram-positive bacteria (3). 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 *Vibrio*'s, sucrose is added as a fermentable carbohydrate. Bromo thymol blue and thymol blue are the pH indicators. The alkaline pH of the medium improves the recovery of *Vibrio cholerae*. Strains of *Vibrio cholerae* produce yellow colonies on TCBS Agar because of fermentation of sucrose. *Vibrio alginolyticus* also produce yellow colonies. *Vibrio parahaemolyticus* is a sucrose non-fermenting organism and produces blue-green colonies, as of *Vibrio vulnificus*. As mentioned previously, occasional isolates of *Pseudomonas* and *Aeromonas* species also produce blue-green colonies, but overall TCBS Agar is highly selective and any H₂S-negative colony is possibly *Vibrio* species. The medium should be inoculated heavily with faecal specimens because some *Vibrio* species readily die off on the medium, owing to fermentation of sucrose and accumulation of acids.

Type of specimen

Clinical : faeces, etc; Food samples; Water samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5).

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (6).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (7).

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.
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.

Quality Control

Appearance

Light yellow to tan coloured homogeneous free flowing powder

Gelling

Firm, comparable with 1.4% Agar gel.

Colour and Clarity of prepared medium

Bluish green coloured clear to slightly opalescent gel forms in petri plates.

Reaction

Reaction of 8.8% w/v aqueous solution at 25°C. pH : 8.6±0.1

pH

8.50-8.70

Cultural Response

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

Organism	Inoculum ((CFU)	Growth	Recovery	Colour of colony
Productivity				
<i>Vibrio parahaemolyticus</i> NCTC 10885 (00185*)	50-100	good-luxuriant	≥50%	blue
<i>Vibrio furnissii</i> NCTC 11218 ((00186*)*)	50-100	good-luxuriant	≥50%	greenish yellow
Specificity				
<i>Escherichia coli</i> ATCC 25922 (00013*)	≥10 ⁴	inhibited	0%	
<i>Escherichia coli</i> ATCC 8739(00012*)	≥10 ⁴	inhibited	0%	
<i>Escherichia coli</i> ATCC 11775(00090*)	≥10 ⁴	inhibited	0%	
Additional Microbiological Testing				
<i>Vibrio parahaemolyticus</i> ATCC 17802 (00037*)	50-100	good-luxuriant	≥50%	blue
<i>Vibrio cholerae</i> ATCC 15748	50-100	good-luxuriant	≥50%	yellow

<i>Vibrio fluvialis</i> ATCC 33809 (00137*)	50-100	good-luxuriant	$\geq 50\%$	yellow
<i>Vibrio vulnificus</i> ATCC 29306	50-100	fair - good	20-30%	greenish yellow
# <i>Proteus hauseri</i> ATCC 13315	$\geq 10^4$	inhibited	0%	
<i>Shigella flexneri</i> ATCC 12022 (00126*)	$\geq 10^4$	inhibited	0%	
<i>Enterococcus faecalis</i> ATCC 29212 (00087*)	$\geq 10^4$	inhibited	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. 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 (4,5).

Reference

1. Nakanishi, 1963, Modern Media, 9:246.
2. Kobayashi, Enomoto, Sakazaki and Kuwahara, 1963, Jap. J. Bacteriol., 18:387.
3. Howard B., 1994, Clinical and Pathogenic Microbiology, 2nd ed., The C.V. Mosby Co., Mosby-Year Book, Inc., St. Louis.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. 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.
6. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods,
7. Lipps WC, Braun-Howland EB, Baxter TE, eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.

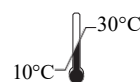
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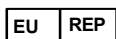
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