TCBS Agar

M870S

Intended Use:

Recommended for selective isolation of *Vibrio cholerae* and other enteropathogenic *Vibrio*'s. The composition and performance criteria of this medium are as per specifications laid down in IS:5887(Part V), APHA, FDA BAM, ISO 21872-1:2017(E), ISO 11133:2014.

Composition**

ISO 21872-1:2017(E), FDA BAM, IS:5887(Part V)

Ingredients	g / L
Yeast extract	5.000
Peptone	10.000
Sodium citrate	10.000
Sodium thiosulphate	10.000
Dried bovine bile	8.000
Sucrose	20.000
Sodium chloride	10.000
Iron III citrate	1.000
Bromo thymol blue	0.040
Thymol blue	0.040
Agar-agar	8.0-18.0
Final pH (at 25°C)	8.6±0.2

TCBS Agar **M870S**

Ingredients	g / L
Yeast extract	5.000
Peptone	10.000
Sodium citrate,dihydrate	10.000
Sodium thiosulphate,pentahydrate	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)	8.6±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Oxgall

Directions

Suspend 84.22 gram (the equivalent weight of dehydrated medium per litre) 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 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. Present formulation is recommended by IS:5887(Part V) (3), APHA (4), FDA-BAM (5), ISO21872-1:2017(E) (6), ISO 11133:2014 (7), for isolation of *Vibrio cholerae* and *Vibrio parahaemolyticus.* Inoculate the sample in Alkaline Peptone Water (M618S), incubate overnight at 35°C. Subculture the growth on TCBS Agar (M870S).

Peptone and yeast extract provide nitrogenous, carbonaceous compounds, long chain amino acids, vitamin B complex and other essential growth nutrients. Bile and the sodium citrate inhibit gram-positive bacteria (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 *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. In case of doubt, confirm *Pseudomonas* using oxidase test. The medium should be incoulated 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

Food samples; Water samples

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (3,5,6,7,9).
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

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 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 H_2S 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 8.42% w/v aqueous solution at 25°C. pH : 8.6±0.2

pH

8.40-8.80

Cultural Response

Productivity : Cultural characteristics observed after an incubation at 37±1°C for 24±3 hours.
Selectivity : Cultural characteristics observed after an incubation at 37±1°C for 24±3 hours.

Organism	Inoculum (CFU)	Growth	Growth	Colour of colony
Productivity				
Vibrio parahaemolyticus NCTC 10885 (00185*)	50-100	good-luxuriant	good-luxuriant	green colonies (sucrose negative)
Vibrio furnissii NCTC 11218 (00186*)	50-100	good-luxuriant	good-luxuriant	yellow colonies (sucrose negative)
Selectivity				
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	inhibited	
Escherichia coli ATCC 8739 (00012*)	>=10^4	inhibited	inhibited	
Escherichia coli ATCC 11775 (00090*)	>=10^4	inhibited	inhibited	

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 inorder 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 (10,11).

Reference

1. Nakanishi Y., 1963, Modern Media 9: 246.
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6. Microbiology of the food chain —Horizontal method for the determination of *Vibrio* spp., Part 1:Detection of potentially enteropathogenic *Vibrio parahaemolyticus*, *Vibrio cholerae* and *Vibrio vulnificus*, ISO 21872-1:2017(E).
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9. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
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