

## Technical Information

### TCBS Agar

**Product Code: DM 1870S**

**Application:** - TCBS Agar is used for selective isolation of *Vibrio cholerae* and other enteropathogenic *Vibrio s.*

### Composition\*\*

Ingredients	Gms / Litre
Yeast extract	5.000
Peptic digest of animal tissue	10.000
Sodium citrate	10.000
Sodium thiosulphate	10.000
Sodium cholate	3.000
Oxgall	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

### Principle & Interpretation

TCBS Agar was first devised by Nakanishi <sup>(1)</sup> which was further modified by Kobayashi et al <sup>(2)</sup>. It promotes rapid growth of pathogenic *Vibrio s* after 24 hours incubation at 37°C and contaminating non- *vibrio s* are suppressed. Present formulation is recommended by BIS <sup>(3)</sup> for isolation of *Vibrio cholerae* and *Vibrio parahaemolyticus*. The sample inoculated in Alkaline Peptone Water (DM1618S), and incubated overnight at 35°C. & further subculture the growth on TCBS Agar (DM1870S).

Peptic digest of animal tissue and yeast extract provide nitrogenous compounds, vitamin B complex and other essential growth nutrients. Oxgall, a derivative of bile salts and the sodium citrate inhibit gram-positive bacteria <sup>(4)</sup>. 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 H<sub>2</sub>S-negative colony is possibly *Vibrio* species. In case of doubt, confirm *Pseudomonas* using oxidase test.

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.

### Methodology

Suspend 89 grams of powder media in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 50°C and pour into sterile Petri plates.

## Quality Control

### Physical Appearance

Light yellow coloured homogeneous free flowing powder

### Gelling

Firm, comparable with 1.5% Agar gel

### Colour and Clarity of prepared medium

Bluish green clear to slightly opalescent gel forms in Petri paltes

### Reaction

Reaction of 8.9% w/v aqueous solution at 25°C. pH : 8.6±0.2

### pH range

8.40-8.80

### Cultural Response/Characteristics

DM 1870S: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
<i>Escherichia coli</i> ATCC 25922	≥10 <sup>3</sup>	inhibited	0%	
<i>Shigella flexneri</i> ATCC 12022	≥10 <sup>3</sup>	inhibited	0%	
<i>Enterococcus faecalis</i> ATCC 29212	≥10 <sup>3</sup>	inhibited	0%	
<i>Vibrio cholerae</i> ATCC 15748	50-100	good-luxuriant	≥50%	yellow
<i>Vibrio fluvialis</i> ATCC 33809	50-100	good- luxuriant	≥50%	yellow
<i>Vibrio parahaemolyticus</i> ATCC 17802	50-100	good- luxuriant	≥50%	blue
<i>Vibrio vulnificus</i> ATCC 29306	50-100	fair-good	30-40%	greenish yellow

## Storage and Shelf Life

**Dried Media:** Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.

**Prepared Media:** 2-8<sup>o</sup> in sealable plastic bags for 2-5 days.

## Further Reading

1. Nakanishi, 1963, Modern MEdia, 9:246.
2. Kobayashi, Enomoto, Sakazaki and Kuwahara, 1963, Jap. J. Bacteriol., 18:387.
3. Bureau of Indian Standards, IS: 5887 (Part V) 1976, reaffirmed 1986.
4. Howard B., 1994, Clinical and Pathogenic Microbiology, 2nd ed., The C.V. Mosby Co., Mosby-Year Book, Inc., St. Louis.

## Disclaimer :

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