# Technical Information

## TCBS Agar (Selective)

## Product Code: DM 1870

**Application**: - TCBS Agar is recommended for the selective isolation of *Vibrio cholerae* and other enteropathogenic *Vibrios.*

## Composition**

| Ingredients | Gms / Litre |
|---|---|
| Peptone, special | 10.000 |
| Yeast extract | 5.000 |
| 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) | 8.8±0.2 |

**Formula adjusted, standardized to suit performance parameters

## Principle & 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 as 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 (DM1618), 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. Oxgall, a derivative of bile salts and sodium citrate inhibit growth of gram-positive bacteria and coliforms [8]. Sodium thiosulphate acts 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. 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 $H_2S$ 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.

## Methodology

Suspend 89.08 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 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 range**

8.60-9.00

**Cultural Response/Characteristics**

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

| Organism | Inoculum (CFU) | Growth | Recovery | Colour of colony |
|---|---|---|---|---|
| *Escherichia coli ATCC 25922* | >=$10^3$ | inhibited | 0% | |
| *Shigella flexneri ATCC 12022* | >=$10^3$ | inhibited | 0% | |
| *Enterococcus faecalis ATCC29212* | >=$10^3$ | inhibited | 0% | |
| *Vibrio cholerae ATCC 15748* | 50-100 | good- luxuriant | >=50% | yellow |
| *Vibrio fluvialis ATCC 33809* | 50-100 | good- luxuriant | >=50% | yellow |
| *Vibrio parahaemolyticus ATCC 17802* | 50-100 | good- luxuriant | >=50% | bluish green |
| *Vibrio vulnificus 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-$8^0$ in sealable plastic bags for 2-5 days.

## Further Reading

1. Kobayashi T., Enomoto S., Sakazaki R., and Kuwahara S., 1963, Jap. J. Bacteriol., 18: 387.
2. Nakanishi Y., 1963, Modern Media 9: 246.
3. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., American Public Health Association, Washington, D.C.
4. Clesceri L. S., Greenberg A. E. and Eaton A. D., (Eds.), 1998, Standard Methods for the Examination of Water and Wastewater, 20th Ed., American Public Health Association, Washington, D.C.
5. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Eds.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
6. Furniss A. L., Lee J. V. and Donovan T. J., 1978, The Vibrios, Public Health Laboratory Service Monograph Series No. 11, Maidstone Public Health Laboratory, H.M.S.O., London, England.
7. Forbes B. A., Sahm A. S. and Weissfeld D. F., 1998, Bailey & Scotts Diagnostic Microbiology, 10th Ed., Mosby, Inc. St. Louis, Mo.
8. Howard B., 1994, Clinical and Pathogenic Microbiology, 2nd Ed., The C.V. Mosby.
9. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams & Wilkins, Baltimore, Md.
10. Morris G. K., Merson M. H., Huq A. K., Kibrya A. K. and Black R., 1979, J. Clin. Microbiol., 9:79

## Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
- The product conform solely to the technical information provided in this booklet and to the best of knowledge research and development work carried at **CDH** is true and accurate
- **Central Drug House Pvt. Ltd.** reserves the right to make changes to specifications and information related to the products at any time.
- Products are not intended for human or animal diagnostic or therapeutic use but for laboratory, research or further manufacturing of diagnostic reagents extra.
- Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.
- Do not use the products if it fails to meet specificatons for identity and performens parameters.