# TCBS Agar

## Intended use
TCBS Agar is a selective medium used for isolating and cultivating *Vibrios* causing cholera and food poisoning from clinical and non-clinical specimens.

## Summary
Thiosulphate Citrate Bile Salts Sucrose (TCBS) Agar is a primary plating medium for the selective isolation of *Vibrios* that cause cholera, diarrhoea and food poisoning. It was developed by Nakanishi *et al.,* and was modified by Kobayashi. The combination of alkaline peptone water and TCBS Agar is used in many procedures for the isolation of *V. cholerae* and other *Vibrio* species from foods, water and faeces. This medium is included in the Bacteriological Analytical Manual for food testing.

## Principle
Proteose peptone and yeast extract provide nitrogenous compounds, vitamin B complex and other essential growth nutrients. Oxgall and sodium citrate inhibits Gram-positive bacteria. Sodium thiosulphate serves as the sulphur source and in combination with ferric citrate detects hydrogen sulphide production. Sucrose is the fermentable carbohydrate for the metabolism of *Vibrios*. The alkaline pH of the medium enhances the recovery of *V. cholerae*. Thymol blue and bromothymol blue are included as indicators of pH change. Sodium chloride maintains the osmotic balance.

## Formula*

| **Ingredients** | **g/L** |
|---|---|
| Sucrose | 20.0 |
| Proteose Peptone | 10.0 |
| Sodium Citrate | 10.0 |
| Sodium Thiosulphate | 10.0 |
| Sodium Chloride | 10.0 |
| Oxgall | 8.0 |
| Yeast Extract | 5.0 |
| Ferric Citrate | 1.0 |
| Thymol Blue | 0.04 |
| Bromothymol Blue | 0.04 |
| Agar | 15.0 |
| Final pH (at 25°C) | 8.6 ± 0.2 |

*Adjusted to suit performance parameters

## Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

## Type of Specimen
Clinical samples; Food samples; Water sample

## Specimen Collection and Handling
Ensure that all samples are properly labelled.
Follow appropriate techniques for handling samples as per established guidelines.
Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure.
The samples must be stored and tested within the permissible time duration.
After use, contaminated materials must be sterilized by autoclaving before discarding.

## Directions

1. Suspend 89.08 g of the powder in 1000 mL purified / distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. DO NOT AUTOCLAVE.
5. Cool to 45°C-50°C and pour into sterile petridishes, Use immediately.

## Quality Control

**Dehydrated Appearance:** Cream to yellow coloured with tan cast, homogeneous, free flowing powder.
**Prepared Appearance:** Green coloured, slightly opalescent gel forms in petridishes.
**Cultural Response:** Cultural characteristics observed after an incubation of 18-24 hours at 30°C-35°C.

| **Organism (ATCC)** | **Growth** | **Colour of Colony** |
|---|---|---|
| *Vibrio cholerae* (14748) | Good | Yellow |
| *Vibrio parahaemolyticus* (MTCC 451) | Good | Blue green |
| *Escherichia coli* (25922) | Complete Inhibition | - |
| *Proteus hauseri* (13315) | Partial Inhibition | Yellow |
| *Streptococcus faecalis* (11420) | Partial Inhibition | Yellow |

## Interpretation of Results

Colony morphology on TCBS Agar is as follows:
*V. cholerae* ----------Yellow colonies
*V. parahaemolyticus* ----------Colonies with blue to green centers
*Proteus/Enterococcus* ---------- Partial inhibition. If growth occurs, colonies are small, yellow and translucent.

## Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

## Precautions/Limitations

1. Cultures grown on TCBS Agar should be examined immediately after removal from the incubator as yellow colonies of *Vibrios*, e.g. *V. cholerae* may revert to a green colour when left at room temperature.
2. The identification of the various *Vibrio* species on TCBS Agar is presumptive and further tests are required for confirmation.
3. Yellow colonies on TCBS Agar will give unsatisfactory oxidase reactions.
4. Initial isolation of *V. parahaemolyticus* may be confused with *Plesiomonas shigelloids* and *Pseudomonas* species.
5. Sucrose fermenting *Proteus* species produce yellow colonies, which may resemble those of *Vibrio*.
6. A few strains of *V. cholerae* may appear green or colourless on TCBS Agar due to delayed.
7. Colonies taken from TCBS Agar are 'sticky' and react poorly in slide agglutination tests. Subculture to Nutrient Agar before slide agglutination tests are carried out.

## Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

## References


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. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

## Product Presentation:

| Cat No. | Product description | Pack Size |
|---|---|---|
| 201200010100 | Dehydrated Culture Media | 100 g |
| 201200010500 | Dehydrated Culture Media | 500 g |