**Technical Data**

**TCBS Agar Plate**

**Intended Use:**
Recommended for the selective isolation of *Vibrio cholerae* and other enteropathogenic *Vibrio's*

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone, special</td>
<td>10.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>5.000</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>10.000</td>
</tr>
<tr>
<td>Sodium thiosulphate</td>
<td>10.000</td>
</tr>
<tr>
<td>Sodium cholate</td>
<td>3.000</td>
</tr>
<tr>
<td>Bile#</td>
<td>5.000</td>
</tr>
<tr>
<td>Sucrose</td>
<td>20.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>10.000</td>
</tr>
<tr>
<td>Ferric citrate</td>
<td>1.000</td>
</tr>
<tr>
<td>Bromo thymol blue</td>
<td>0.040</td>
</tr>
<tr>
<td>Thymol blue</td>
<td>0.040</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>8.8±0.2</td>
</tr>
</tbody>
</table>

**Directions**

Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate.

**Principle And Interpretation**

TCBS Agar was developed by Kobayashi et al (7), who modified the selective medium of Nakanishi (11). Although this medium was originally designed for the isolation of *V.cholerae* and *V.parahaemolyticus*, most Vibrios grow to healthy large colonies with many different colonial morphologies. TCBS Agar is also recommended by APHA for the selective isolation of *V.cholerae* and *V.parahaemolyticus* (1,12). Enrichment in Alkaline Peptone Water (M618), followed by isolation on TCBS Agar is routinely used for isolation of *V.cholerae* (2,3,10). 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. Bile and sodium citrate inhibit gram-positive bacteria and coliforms (4). 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 Vibrios, sucrose is added as a fermentable carbohydrate. *Vibrio* that is able to utilize sucrose will form yellow colonies. Bromothymol blue and thymol blue are the pH indicators. The alkaline pH of the medium improves the recovery of *V cholerae*. Strains of *V.cholerae* produce yellow colonies on TCBS Agar because of fermentation of sucrose. *V.alginolyticus* also produce yellow colonies. *V.parahaemolyticus* is a sucrose non-fermenting organism and therefore produces blue-green colonies, as does *V.vulnificus*. A few strains of *V.cholerae* may appear green or colourless on TCBS Agar due to delayed sucrose fermentation.(8).

**Type of specimen**

Clinical : faeces; Food samples; Water samples

**Specimen Collection and Handling**

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6). For food samples, follow appropriate techniques for sample collection and processing as per guidelines (12). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (1). After use, contaminated materials must be sterilized by autoclaving before discarding.

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Please refer disclaimer Overleaf.
**Warning and Precautions**

In Vitro diagnostic use. 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. (8).
3. *Proteus* species that are sucrose-fermenters may form yellow colonies (8).
4. TCBS Agar is not a suitable medium for oxidase testing of *Vibrio* species (9).
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. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
8. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user’s unique requirement.
9. Further biochemical and serological tests must be carried out for complete identification.
10. It is recommended to store the plates at 24-30°C to avoid minimum condensation.

**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**
Sterile TCBS Agar in 90 mm disposable Petri plates.

**Colour**
Bluish green coloured medium

**Quantity of medium**
25ml of medium in 90mm disposable plate

**Reaction**
8.60-9.00

**Sterility test**
Passes release criteria

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

<table>
<thead>
<tr>
<th>Organism</th>
<th>Growth</th>
<th>Inoculum (CFU)</th>
<th>Recovery</th>
<th>Colour of colony</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Vibrio parahaemolyticus ATCC 17802 (00037</em>)*</td>
<td>good-luxuriant</td>
<td>50-100</td>
<td>&gt;=50%</td>
<td>bluish green</td>
</tr>
<tr>
<td><em>Vibrio vulnificus ATCC 29306</em></td>
<td>fair-good</td>
<td>50-100</td>
<td>&gt;=30%</td>
<td>greenish yellow</td>
</tr>
<tr>
<td><em>Vibrio fluvialis ATCC 33809 (00137</em>)*</td>
<td>good-luxuriant</td>
<td>50-100</td>
<td>&gt;=50%</td>
<td>yellow</td>
</tr>
<tr>
<td><em>Vibrio cholerae ATCC 15748</em></td>
<td>good-luxuriant</td>
<td>50-100</td>
<td>&gt;=50%</td>
<td>yellow</td>
</tr>
</tbody>
</table>
**Storage and Shelf Life**

On receipt store between 20-30°C Use before expiry date on the label. 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 (5,6).

**Reference**


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**Escherichia coli ATCC 25922 (00013*)**

- Inhibited
- >=10³
- 0%

**Enterococcus faecalis ATCC 29212 (00087*)**

- Inhibited
- >=10³
- 0%

**Shigella flexneri ATCC 12022 (00126*)**

- Inhibited
- >=10³
- 0%

Key: (*) Corresponding WDCM numbers
In vitro diagnostic medical device

CE Marking

Storage temperature

Do not use if package is damaged

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