**TCBS Agar**

**Intended Use:**
Recommended for the selective isolation and cultivation of *Vibrio cholerae* and other enteropathogenic *Vibrio's* causing food poisoning from clinical and food specimen.

**Composition**

**Ingredients**  |  **Gms / Litre**
---|---
Proteose peptone  |  10.000
Yeast extract  |  5.000
Sodium thiosulphate  |  10.000
Sodium citrate  |  10.000
Bile  |  8.000
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

**Directions**
Suspend 89.08 grams in 1000 ml purified/ 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 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 *Vibrio's* 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* (2,12). Enrichment in Alkaline Peptone Water (M618), followed by isolation on TCBS Agar is routinely used for isolation of *V.cholerae* (3,4,10).

Proteose peptone and yeast extract provide nitrogenous compounds, vitamin B complex and other essential growth nutrients. Bile, a derivative of bile salts and sodium citrate inhibit gram-positive bacteria and coliforms (5). 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. *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*. 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*.

**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 (6,10).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,12,13). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(2)
After use, contaminated materials must be sterilized by autoclaving before discarding.
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 (8).
6. TCBS Agar is highly selective for *Vibrio* species. Any H₂S 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 8.9% w/v aqueous solution at 25°C. pH : 8.6±0.2

pH
8.40-8.80

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

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
<th>Colour of colony</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Shigella flexneri ATCC 12022</em></td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td><em>Vibrio cholerae ATCC 15748</em></td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>yellow</td>
</tr>
<tr>
<td><em>Vibrio fluvialis ATCC 33809</em></td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>yellow</td>
</tr>
<tr>
<td><em>Vibrio parahaemolyticus ATCC 17802</em></td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>bluish green</td>
</tr>
<tr>
<td><em>Vibrio vulnificus ATCC 29307</em></td>
<td>50-100</td>
<td>fair-good</td>
<td>&gt;=30%</td>
<td>greenish yellow</td>
</tr>
<tr>
<td><em>Enterococcus faecalis ATCC 29212</em></td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli ATCC 25922</em></td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td><em>Proteus vulgaris ATCC 13315</em></td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
</tr>
</tbody>
</table>

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 in order 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. 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 (6,10).

Reference

Disclaimer:

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