TCBS HiCynth™ Agar (Selective)

Intended Use:
Recommended for 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>HiCynth™ Peptone No.3*</td>
<td>16.000</td>
</tr>
<tr>
<td>HiCynth™ Peptone No.5*</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>Synthetic detergent</td>
<td>2.000</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>

**Formula adjusted, standardized to suit performance parameters

* Chemically defined peptones

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 Nakanishii (10). 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* (1,11). Enrichment in Alkaline Peptone Water / Alkaline HiCynth™ Peptone Water (M618/MCD618), followed by isolation on TCBS Agar is routinely used for isolation of *V.cholerae* (2,3,6) . TCBS HiCynth™ Agar is prepared by replacing animal and vegetable peptones with chemically defined peptones to avoid BSE/TSE risks associated with animal peptones. HiCynth™ Peptone No.3 and HiCynth™ Peptone No.5 provide nitrogenous compounds, vitamin B complex and other essential growth nutrients. Synthetic detergent 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 HiCynth™ 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*. *Proteus* species that are sucrose-fermenters may form yellow colonies (8). TCBS HiCynth™ Agar is a suitable medium for oxidase testing of Vibrio species (9). A few strains of *V. cholerae* may appear green or colourless on TCBS HiCynth™ Agar due to delayed sucrose fermentation (8). TCBS HiCynth™ Agar is highly selective for *Vibrio* species. However, occasional isolates of *Pseudomonas* and *Aeromonas* may also form blue green colonies on TCBS Agar (8). Any *H₂S* 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.
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 (11).
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.

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.
3. Proteus species that are sucrose-fermenters may form yellow colonies.
4. TCBS Agar is not a suitable medium for oxidase testing of Vibrio species.
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. 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 : 8.8±0.2
pH
8.60-9.00
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>Escherichia coli ATCC 25922 (00013*)</td>
<td>&gt;10⁴</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
</tr>
</tbody>
</table>

Please refer disclaimer Overleaf.
**Vibrio parahaemolyticus ATCC 17802 (00037*)**

50-100 good-luxuriant >=50%

bluish green

**Vibrio vulnificus ATCC 29306**

50-100 fair-good >=30%

greenish yellow

**Vibrio fluvialis ATCC 33809 (00137*)**

50-100 good-luxuriant >=50%

yellow

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

>=10⁴ inhibited 0%

**Vibrio cholerae ATCC 15748**

50-100 good-luxuriant >=50%

yellow

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

>=10⁴ inhibited 0%

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 inorder 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. 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

In vitro diagnostic medical device

CE Marking

Storage temperature

10°C - 30°C

Do not use if package is damaged

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