ITC Broth Base (TTC Broth Base)

Intended Use:
Recommended for selective enrichment and enumeration of Yersinia enterocolitica.

Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptone</td>
<td>10.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>1.000</td>
</tr>
<tr>
<td>Magnesium chloride hexahydrate</td>
<td>60.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Malachite green</td>
<td>0.010</td>
</tr>
<tr>
<td>Triclosan (Irgasan)</td>
<td>0.001</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>6.9±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters

Directions
Suspend 44.11 grams (the equivalent weight of dehydrated medium per litre) in 988 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add rehydrated contents of 1 vial of Ticarcillin Supplement (FD102) and Potassium Chlorate Supplement (FD103). Mix well before dispensing in sterile tubes or flasks as desired.

Principle And Interpretation
The genus Yersinia belongs to the family Enterobacteriaceae. They are usually nitrate reductase positive and show fermentative metabolism. The genus comprises of 11 species, of which Yersinia enterocolitica is most important as a causative agent of human foodborne illness. Variety of enrichment methods has been described for recovery of Y.enterocolitica from foods. The most efficient procedures for recovering enteropathogenic bacteria from foods have incorporated at least one and often two enrichment steps before plating onto selective differential agar media. ITC Broth is formulated in accordance with APHA (7) and is recommended by ISO Committee (3) as a selective enrichment medium for Y.enterocolitica from foods. ITC Broth was developed by Wauters et al (8) as a new enrichment broth, derived from modified Rappaport Broth and based on the selective agents irgasan, ticarcillin and potassium chlorate.

Tryptone and yeast extract provide nitrogeneous and carbonaceous compounds, lond chain amino acids and other essential growth nutrients. Ticarcillin has inhibitory action on both gram-positive and gram-negative organisms. Irgasan inhibits gram-positive organisms. Potassium chloride has disinfecting properties.

For enrichment prepare 1: 10 homogenate of food sample by weighing 25 grams of food and adding it to 225 ml of primary enrichment medium. Prepare homogenate and carefully transfer the homogenate into sterile jar for incubation. After incubation, streak onto agar plates such as MacConkey Agar (M081). After incubation, observe for the colonies of Yersinia, which are pinkish coloured, smooth and have an entire edge. Colonies of Yersinia are larger on agar media when incubated at 25°C as Y.enterocolitica is more active biochemically at 25°C than at 35-37°C.

Type of specimen
Clinical samples - Faeces; Food and dairy samples; Water samples

Specimen Collection and Handling:
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,6,9).
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.

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 blue homogeneous free flowing powder

Colour and Clarity of prepared medium
Peacock green coloured, clear solution without any precipitate

Reaction
Reaction of 4.41% w/v aqueous solution at 25°C. pH : 6.9±0.2

pH
6.70-7.10

Cultural Response
Cultural characteristics observed with added Ticarcillin Supplement (FD102) and Potassium Chlorate Supplement (FD103) after an incubation at 25-30°C for 24-48 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> ATCC 25922 (00013*)</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> subsp. aureus ATCC 25923 (00034*)</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em> ATCC 27729</td>
<td>50-100</td>
<td>good-luxuriant</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 2-8°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 (4,5).

Reference

Please refer disclaimer Overleaf.