Enterococcus Confirmatory HiCynth™ Agar

Intended Use:
Recommended for confirming the presence of Enterococci in water supplies and other sources.

Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>HiCynth™ Peptone No.4*</td>
<td>5.000</td>
</tr>
<tr>
<td>HiCynth™ Peptone No.5*</td>
<td>5.000</td>
</tr>
<tr>
<td>Dextrose (Glucose)</td>
<td>5.000</td>
</tr>
<tr>
<td>Sodium azide</td>
<td>0.400</td>
</tr>
<tr>
<td>Methylene blue</td>
<td>0.010</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>Final pH ( at 25°C)</td>
<td>8.0±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters

* Chemically defined peptones

Directions
Suspend 30.41 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Dispense in tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the agar tubes to cool to 45-50°C in a slanted position.

Principle And Interpretation
Enterococcus Confirmatory Agar formulated by Sandholzer and Winter (5) is used for the detection of Enterococci in water supplies, swimming pools, sewage etc. Enterococci are found as normal flora in the gastrointestinal tracts of humans and animals. They are becoming increasingly important agents of human diseases largely because of their resistance to antimicrobial agents to which other Streptococci are generally susceptible (2). The Enterococcus is a subgroup of the fecal Streptococci group that includes Enterococcus faecalis, Enterococcus faecium, Enterococcus gallinarum, and Enterococcus avium (1). Enterococci are differentiated from other Streptococci by their ability to grow in 6.5% sodium chloride, at pH 9.6 and at 10°C and 45°C (1).

The ability of organisms to grow in the presence of variable amounts of sodium chloride is a test that has been used to characterize several bacteria, including the viridans Streptococci. It is useful for presumptive identification of the Enterococcal group D organisms which have the specific ability to grow in the presence of 6.5% NaCl incorporated into the medium. A positive test is the presence of bacterial growth in the medium. If the organism is bile esculin positive and grows in 6.5% NaCl broth, the organism is an Enterococcus species and if the organism is bile esculin positive and fails to grow in the 6.5%NaCl broth, the organism belongs to a group D Streptococci. The enterococcal portion of the faecal Streptococcus group is a valuable bacterial indicator for determining the extent of faecal contamination of recreational surface waters (1).

Enterococcus Confirmatory 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.4, HiCynth™ Peptone No.5, dextrose provide carbonaceous, nitrogenous compounds, long chain amino acids, vitamins and other essential growth nutrients for the growth of Enterococci. Sodium azide inhibits other contaminating flora. The positive presumptive tests are confirmed by inoculating from Enterococcus Presumptive Broth (M419) to Enterococcus Confirmatory slant-broth combination prepared with an Azide Agar medium (Enterococcus Confirmatory HiCynth™ Agar, MCD392) overlaid with a Salt Azide Penicillin Broth (Enterococcus Confirmatory Broth, M394). A negative catalase test is considered confirmed positive evidence of the presence of Enterococci. Single strength medium can be used for small inoculum. Production of acid and turbidity in an azide presumptive broth when incubated at 45°C is considered positive presumptive evidence for the presence of Enterococci, which is confirmed by inoculating on Confirmatory Agar (MCD392).

Type of specimen
Water samples
Specimen Collection and Handling

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
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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. 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 yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Light blue coloured clear to slightly opalescent gel forms in tubes as slants

Reaction
Reaction of 3.04% w/v aqueous solution at 25°C. pH: 8.0±0.2

pH
7.80-8.20

Cultural Response
Cultural characteristics observed after an incubation at 45°C for 18-24 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli ATCC 25922</em></td>
<td>&gt;=10^4</td>
<td>inhibited</td>
</tr>
<tr>
<td><em>Enterococcus faecalis ATCC</em></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 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. 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Please refer disclaimer Overleaf.
Reference

5. Sandholzer and Winter, 1946, Commercial Fisheries Leaflet T1a

Disclaimer:
User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.