C.L.E.D. HiCynth™ Agar w/BTB

Intended use

Recommended for isolation, enumeration and identification of urinary pathogens on the basis of lactose fermentation.

Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>HiCynth™ Peptone No.1*</td>
<td>11.000</td>
</tr>
<tr>
<td>Lactose</td>
<td>10.000</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>0.128</td>
</tr>
<tr>
<td>Bromothymol blue</td>
<td>0.020</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.3±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters
*Chemically defined peptone

Directions

Suspend 36.15 grams in 1000 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. Mix well and pour into sterile Petri plates.

Principle And Interpretation

On a solid medium, Sandys reported that swarming of *Proteus* species can be controlled by restricting the electrolytes (8). Formerly swarming of Proteus was controlled by adding alcohol, surface-active agent, sodium azide, boric acid etc. to the medium (8). Later on Sandy's medium was modified by Mackey and Sandys (6), by replacing mannitol by lactose and sucrose and elevating concentration of agar and bromothymol blue. This formulation was further modified by the same authors, called C.L.E.D. (Cystine-Lactose-Electrolyte-Deficient) by deleting the sucrose and by including L-cystine for promoting the growth of cystine dependent dwarf colony coliforms (7). C.L.E.D. HiCynth™ Agar is modified by using chemically defined peptones replacing animal or vegetable peptones to avoid BSE/TSE risks associated with animal peptones. This medium is recommended for use in urinary bacteriology, promoting the growth of all urinary pathogens. C.L.E.D. Medium is also recommended for dip stick procedures and as dip inoculum transport medium for urine specimens (2, 6, 7).

HiCynth™ peptone No.1 provides nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other essential growth nutrients. Lactose is the fermentable sugar. L-cystine supports the growth of dwarf coliform colony. Bromothymol blue is the pH indicator which turns yellow at acidic pH. Bacteriuria may be quantitated by inoculating the surface of an agar medium by proper dilution. Inoculate the medium immediately after urine collection. It can also be inoculated by calibrated loop or duplicate dilution pour plate methods (1, 5).

Type of specimen

Clinical samples - urine

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions:

In Vitro diagnostic Use only. 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. Initiation of antibiotic therapy, before collection of sample, low urine pH (less than 5) etc. may result in low bacterial count from infected patients.

2. *Shigella* species may not grow on this medium.

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
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Green coloured, clear to slightly opalescent gel forms in Petri plates.

Reaction
Reaction of 3.61% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH
7.10-7.50

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>Enterococcus faecalis</em> ATCC 29212 (00087*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=70%</td>
<td>slight yellowish or greenish</td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 25922 (00013*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=70%</td>
<td>yellow, opaque, centre slightly deeper yellow</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em> ATCC 13883 (00097*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=70%</td>
<td>yellow to whitish blue blue</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em> ATCC 13315</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=70%</td>
<td>blue</td>
</tr>
<tr>
<td><em>Salmonella Typhi</em> ATCC 6539</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=70%</td>
<td>bluish</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> subsp. <em>aureus</em> ATCC 25923 (00034*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=70%</td>
<td>deep yellow</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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

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.