Buffered Charcoal Yeast Extract HiCynth™ Agar Base

**Intended Use**
Recommended for selective isolation and cultivation of *Legionella* species from cooling towers, clinical and other materials.

**Composition**

**Ingredients**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>HiCynth™ Peptone No.5*</td>
<td>10.000</td>
</tr>
<tr>
<td>Charcoal activated</td>
<td>2.000</td>
</tr>
<tr>
<td>ACES buffer</td>
<td>10.000</td>
</tr>
<tr>
<td>α-Ketoglutarate monopotassium salt</td>
<td>1.000</td>
</tr>
<tr>
<td>Agar</td>
<td>17.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>6.9±0.2</td>
</tr>
</tbody>
</table>

**Directions**
Suspend 20 grams in 500 ml purified / distilled water. Add 2.4 grams KOH pellets and mix to dissolve. 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 sterile rehydrated contents of 1 vial each of Legionella Supplement (FD041A and FD040). Mix well and pour with constant stirring to ensure that charcoal particles get evenly distributed. For additional selectivity, Legionella Selective Supplements (FD017, FD037, FD038) may be added to molten medium as per choice.

**Principle And Interpretation**
Feeley et al (6) originally formulated Charcoal Yeast Extract (CYE) Agar. This medium was a modification of the existing F-G Agar (5). F-G Agar had starch and casein enzymic hydrolysate as ingredients in the composition. Feely et al (5,6) replaced these two with charcoal and yeast extract respectively, and reported better recovery of *Legionella pneumophilla*. Later Paseulle (11) reported that supplementation of the Charcoal Yeast Agar with ACES buffer improved the performance of the medium. Edelstein (4) further modified the medium by adding alpha-ketoglutarate. This addition helped in improving the sensitivity of the medium. Buffered Charcoal Yeast Extract Agar Base is based on Edelsteins Modification.

*Legionella* species are non-spore forming, narrow, gram-negative rods. *Legionella* causes pneumonia (Legionnaires disease) (2) or a milk, febrile disease (Pontiac fever). They do not oxidize or ferment carbohydrates in conventional media or grow on sheep blood agar. Growth is much better and more rapid on Buffered Charcoal Yeast Extract Agar (5,9). Amino acids are the major sources of energy for *Legionella*. The amino acid L-cystine holds an absolute requirement as it plays major role in growth metabolism of *Legionella* (7). This amino acid as well as ferric pyrophosphate helps for the growth of *Legionella*. The media contains charcoal, which acts as a detoxicant. Buffered Charcoal Yeast Extract HiCynth™ Agar Base is prepared by replacing animal and vegetable peptones with chemically defined peptones to avoid BSE/TSE risks associated with animal peptones. HiCynth™ Peptone No.5 acts as a rich source of vitamins, nitrogen as well as carbon. ACES Buffer maintains optimal pH for growth while L-cystine hydrochloride; ferric pyrophosphate and α-ketoglutarate stimulate growth of *Legionella* species. For selective isolation, antibiotic supplements can be used to suppress contaminating microorganisms. Legionella Selective Supplement II (CCVC) (FD037) containing cephalothin, colistin, vancomycin and cycloheximide (3) or Legionella Selective Supplement IV (MWY) (FD040) containing glycine, polymyxin B, anisomycin, vancomycin, bromothymol blue and bromocresol purple (12) are often used. Wear gown, mask and gloves while handling *Legionella* cultures. Work in a safety hood.

**Type of specimen**
Clinical samples - Urine; Water samples

**Specimen Collection and Handling**

Please refer disclaimer Overleaf.
HiMedia Laboratories  

Technical Data

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (8,10). 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. Further biochemical confirmation has to 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**
Grey to black homogeneous free flowing powder

**Gelling**
Firm, comparable with 1.7% Agar gel.

**Colour and Clarity of prepared medium**
Grey-black coloured opalescent gel forms in Petri plates.

**Reaction**
Reaction of 4.0% w/v aqueous solution at 25°C. pH : 6.9±0.2

**pH**
6.70-7.10

**Cultural Response**
Cultural characteristics observed in 90% humid atmosphere with added Legionella Supplement(FD041A and FD040), after an incubation at 35-37°C for 3-4 days.

<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>Escherichia coli</em> ATCC 25922</td>
<td>50-100</td>
<td>none-poor</td>
<td>&lt;10%</td>
<td></td>
</tr>
<tr>
<td><em>(00013</em>)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Legionella dumoffii</em> ATCC 33343</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;50%</td>
<td>light blue-grey</td>
</tr>
<tr>
<td><em>Legionella pneumophila</em> ATCC 33153</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;50%</td>
<td>white grey to blue grey</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>50-100</td>
<td>none-poor</td>
<td>&lt;10%</td>
<td></td>
</tr>
<tr>
<td><em>ATCC 12228</em> (00036*)</td>
<td></td>
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</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.

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 (8,10).

Please refer disclaimer Overleaf.
Reference