Differential Buffered Charcoal Yeast Extract Agar Base

Intended use

Differential Buffered Charcoal Yeast Extract Agar is used for selective isolation and differentiation of *Legionella* species.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast extract</td>
<td>10.000</td>
</tr>
<tr>
<td>Charcoal activated</td>
<td>1.500</td>
</tr>
<tr>
<td>L-Cysteine hydrochloride</td>
<td>0.400</td>
</tr>
<tr>
<td>Ferric pyrophosphate, soluble</td>
<td>0.250</td>
</tr>
<tr>
<td>ACES buffer</td>
<td>10.000</td>
</tr>
<tr>
<td>Alpha - Ketoglutarate</td>
<td>0.200</td>
</tr>
<tr>
<td>Bromocresol purple</td>
<td>0.010</td>
</tr>
<tr>
<td>Bromothymol 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>6.9±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters**

**Directions**

Suspend 37.37 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs (121°C) pressure for 15 minutes. Cool to 45-50°C. If desired aseptically add the rehydrated contents of one vial of FD349 (V.P. Supplement). Mix well and pour into sterile Petri plates.

**Principle And Interpretation**

*Legionella pneumophila* is a gram-negative rod responsible for Legionnaires disease. It infects the respiratory passage when airborne droplets of water are inhaled. In nature, the bacterium lives within the cytoplasm of the waterborne protozoan *Hartmanella* (1).

Common sources of *Legionella* include cooling towers used in industrial cooling water systems as well as in large central air conditioning systems, domestic hot water systems, fountains, and similar disseminators that draw upon a public water supply. Natural sources include freshwater ponds and creeks (2). Initially F-G Agar developed by Feeley et al (3) was used for the isolation of *L. pneumophila*. F-G Agar was further modified by replacing beef extract and casein hydrolysate by yeast extract. Also starch was replaced by activated charcoal (4). The modified F-G Agar was improved by the addition of ACES Buffer (N-2-acetamido-2-aminoethane sulphonic acid) (5). Sensitivity of the resulting Buffered Charcoal Yeast Extract Agar was increased by the addition of alpha-ketoglutarate (6). Differential Buffered Charcoal Yeast Extract Agar Base used for the selective isolation and differentiation of *Legionella* species is based on the formulation of Vickers (7) containing the two dyes, bromocresol purple and bromothymol blue.

The medium contains yeast extract, which provide necessary nutrients for bacterial growth. Ferric pyrophosphate, L-cysteine hydrochloride and alpha- Ketoglutarate stimulates the growth of *Legionella* species (6). Toxic metabolic products produced in the medium get neutralized by activated charcoal which modifies the surface tension of the medium. Bromocresol purple and bromothymol blue help in the identification of *Legionella* species based on colour and colony morphology (8). Polymyxin B inhibits most of the gram-negative bacilli while vancomycin suppresses the growth of most of the gram-positive bacteria. ACES buffer helps to buffer the medium.

**Type of specimen**

Clinical samples - Blood; Water samples

**Specimen Collection and Handling:**

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (9,10).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards. 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. The test sample should be cultured as soon as possible. Culture swabs can be directly streaked on the plate.
2. *Legionella* growth is usually visible within 3-4 days but some species may take up to 2 weeks to appear.
3. Further biochemical confirmation has to be carried out for further confirmation.

**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 grey to black homogeneous free flowing powder

**Gelling**
Firm, comparable with 1.5% Agar gel

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

**Reaction**
Reaction of 3.74% w/v aqueous solution at 25°C. pH: 6.9±0.2

**pH**
6.70-7.10

**Cultural Response**
M814: Cultural characteristics observed with added 50 units/ml Polymyxin B and 1mg/ml Vancomycin, after an incubation at 35-37°C for 72-96 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Growth</th>
<th>Colour of colony</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Legionella dumoffii</em> ATCC 33343</td>
<td>luxuriant</td>
<td>blue-grey</td>
</tr>
<tr>
<td><em>Legionella pneumophila</em> ATCC 33153</td>
<td>luxuriant</td>
<td>white-grey to blue-grey</td>
</tr>
</tbody>
</table>

**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 the 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 (9,10).

**Reference**

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Please refer disclaimer Overleaf.