Letheen Broth

**Intended use**
Letheen Broth is recommended for determination of bactericidal activity of quaternary ammonium compounds using *Escherichia coli* or *Staphylococcus aureus* in accordance with United States Pharmacopoeia.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptamin</td>
<td>10.000</td>
</tr>
<tr>
<td>HM Peptone B #</td>
<td>5.000</td>
</tr>
<tr>
<td>Polysorbate 80</td>
<td>5.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Lecithin</td>
<td>0.700</td>
</tr>
</tbody>
</table>

# - Equivalent to Beef extract

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

**Directions**
Suspend 25.7 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Dispense into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

**Principle And Interpretation**
Glucose Extract Agar was developed according to APHA (1) for use in the microbiological examination of water. Weber and Black (8) modified it further with addition of lecithin and polysorbate 80 and developed a laboratory procedure for evaluating bactericidal activity of quaternary ammonium compounds proposed for sanitizing food utensils (7). Letheen media are recommended by USP in disinfectant challenge testing (6).

HM Peptone B and peptone, supply nitrogenous compounds, carbon, sulphur and other trace elements to the organisms. Lecithin and polysorbate 80 enables the recovery of bacteria from solutions containing residues of disinfectant used in sanitization of utensils and equipment. Lecithin neutralizes quaternary ammonium compounds and polysorbate 80 neutralizes phenolic disinfectants, hexachlorophene and formalin (2,5).

Dehydrated medium may appear moist with brown sugar appearance, does not indicate deterioration.

**Type of specimen**
Water samples, Pharmaceutical samples

**Specimen Collection and Handling:**
For pharmaceutical samples follow appropriate techniques for handling specimens as per established guidelines (6).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (7).

**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 tests must be carried out for 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**
Off-white to yellow homogeneous free flowing powder

**Colour and Clarity of prepared medium**
Light yellow coloured clear to slightly opalescent solution

**Cultural Response**
MU165: Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

<table>
<thead>
<tr>
<th>Organism (ATCC)</th>
<th>Inoculum(CFU)</th>
<th>Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus ATCC 6538 (00032*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
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<td>Staphylococcus aureus ATCC 6538 (00032*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
</tr>
<tr>
<td>Escherichia coli ATCC 8739 (00012*)</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 (3,4).

**Reference**

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