Antibiotic Assay Medium No. 5

Antibiotic Assay Medium No. 5 is used for microbiological assay of Dihydrostreptomycin using *Bacillus subtilis* in accordance with United States Pharmacopoeia.

### Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>6.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>3.000</td>
</tr>
<tr>
<td>Meat extract B #</td>
<td>1.500</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>pH after sterilization</td>
<td>7.9±0.1</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters

# Equivalent to Beef extract

### Directions

Suspend 25.5 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.

Advice: Recommended for the Microbiological assay of Dihydrostreptomycin.

### Principle And Interpretation

This medium is used in the assay of commercial preparations of antibiotics, as well as for antibiotics in body fluids, feeds etc. Medium composition is in accordance to the specifications detailed in the USP, FDA (1,2) and numerically identical to the name assigned by Grove and Randall (3).

Peptone, yeast and meat extract B provides necessary growth nutrients for the test organisms like *Bacillus subtilis*. This medium provides solidified substratum for growth of organisms. The pH 7.9 maintained in this medium provides optimum growth conditions for *Bacillus subtilis* (4). This medium is used to prepare the base as well as seed layer in the microbiological assay of Dihydrostreptomycin.

To perform the antibiotic assay, the Base Agar should be prepared on the same, inoculated with the standardized test culture can be overlaid. Even distribution of the layer is important.

### Quality Control

**Appearance**
Cream to yellow coloured homogeneous free flowing powder

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

**Colour and Clarity of prepared medium**
Medium amber coloured slightly opalescent gel forms in Petri plates.

**pH**
7.80-8.00

**Cultural Response**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Antibiotics assayed</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus subtilis</em> ATCC 6633</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>Dihydrostreptomycin</td>
</tr>
</tbody>
</table>

**Storage and Shelf Life**
Store below 30°C in tightly closed container and use freshly prepared medium. Use before expiry date on the label.

Please refer disclaimer Overleaf.
Reference

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