Antibiotic Assay Medium No. 10

Antibiotic Assay Medium No. 10 is used as seed layer for antibiotic plate assay of Carbenicillin, Colismethate sodium, Colistin and Polymyxin B in accordance with United States Pharmacopoeia.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptone #</td>
<td>17.000</td>
</tr>
<tr>
<td>Soya peptone ##</td>
<td>3.000</td>
</tr>
<tr>
<td>Dextrose</td>
<td>2.500</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Dibasic potassium phosphate</td>
<td>2.500</td>
</tr>
<tr>
<td>Agar</td>
<td>12.000</td>
</tr>
<tr>
<td>pH after sterilization</td>
<td>7.2±0.1</td>
</tr>
</tbody>
</table>

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

# Pancreatic digest of casein

## Papaic digest of soybean

**Directions**

Suspend 42 grams in 1000 ml purified/distilled water containing 10 ml of Polysorbate 80. 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**

This medium is used as seed agar for assay of Polymyxin B, Colistimethate sodium, Colistin and Carbenicillin. The medium composition is in accordance to USP and CFR (1,2) and numerically identical with the name assigned by Groove and Randall (3).

Combination of tryptone and soya peptone provide essential nutrients for the growth of test organisms. Dextrose provides the carbon source, enhances the growth of test organism. Phosphates in the medium enhances buffering action and sodium chloride maintains osmotic equilibrium. Polymixins are reported to have slow diffusion in agar giving smaller zone of inhibition (4). Hence the reduced agar concentration (1.2%) in this medium improves the diffusion of polymixin in the medium. Polysorbate 80 is reported to function synergistically with polymixins on spheroplasts of *Pseudomonas aeruginosa*. Polysorbate 80 enhances the penetration of Polymyxin to the cytoplasmic membrane and hence is an appropriate ingredient in the medium used for assay of Polymyxin (5).

Freshly prepared plates should be used for antibiotic assays. Test organisms are inoculated in sterile seed agar pre-cooled to 40-45°C and spread evenly over the surface of solidified base agar. All conditions in the microbiological assay must be controlled carefully. The use of standard culture media in the test is one of the important steps for good results.

**Quality Control**

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

**Gelling**
Firm, comparable with 1.2% Agar gel.

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

**Reaction**
Reaction of 4.2% w/v aqueous solution containing 1% polysorbate 80 (after sterilization). pH : 7.2±0.1

**pH**
7.10-7.30
Growth Promotion Test
As per United States Pharmacopoeia

Cultural Response
Cultural characteristics observed after an incubation at 32-37°C for 18-24 hours.

Cultural Response

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
<th>Antibiotics assayed</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bordetella bronchiseptica</em></td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
<td>Colistimethate sodium, Colistin, Polymyxin B</td>
</tr>
<tr>
<td>ATCC 4617</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
<td>Carbenicillin</td>
</tr>
<tr>
<td>ATCC 25619</td>
<td></td>
<td></td>
<td></td>
<td></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.

Reference

Revision: 02 / 2015

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