Antibiotic Assay Medium No.9

Antibiotic Assay Medium No.9 is used as Base layer for plate assay of Carbenicillin, Colistimethate sodium 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>Pancreatic digest of casein</td>
<td>17.000</td>
</tr>
<tr>
<td>Papaic digest of soybean</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>20.000</td>
</tr>
<tr>
<td>pH after sterilization</td>
<td>7.2±0.1</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 50 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.

Principle And Interpretation

This medium is widely recommended for assay of Polymyxin B, Colistimethate sodium and Colistin using *Bordetella bronchiseptica* as test organisms. Carbenicillin assay is also performed using this medium with *Pseudomonas aeruginosa*. The medium is formulated in accordance with USP and CFR (1,2) and numerically identical with the name assigned by Groove and Randall (3).

Pancreatic digest of casein and papaic digest of soybean meal serves as source for essential nutrients. Dextrose stimulates the growth by providing carbon and energy. Phosphates in the medium enhance buffering action and sodium chloride maintains osmotic equilibrium in the medium. Agar concentration provides control over the diffusion activity of Polymyxin B antibiotics and provides solid substratum to support the seed agar layer.

To perform the antibiotic assay the Base Agar should be prepared on the same day as the test. For the cylinder method, a base layer of 21 ml is required. Once the base medium has solidified, seed layer inoculated with the standardized 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 2.0% agar gel.

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

pH
7.10-7.30

Cultural Response
MU147: Cultural characteristics observed after an incubation at 36-37.5°C for 18-24 hours.

<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></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Bordetella bronchiseptica  
**ATCC 4617**  
50-100 luxuriant >=50% Polymyxin B, Colistimethate sodium, Colistin

Pseudomonas aeruginosa  
**ATCC 25619**  
50-100 luxuriant >=70% Carbenicillin

**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**  

**Disclaimer:**  
User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory,diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.