Antibiotic Assay Medium B

Intended use

Antibiotic Assay Medium B is used for the microbiological assay of Colistimethate sodium sulphate using *Bordetella bronchiseptica* and *Escherichia coli* in accordance with British 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>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate</td>
<td>2.500</td>
</tr>
<tr>
<td>Glucose monohydrate</td>
<td>2.500</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>pH after sterilization</td>
<td>7.3±0.1</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters

$- $ Pancreatic digest of casein

# - Papaic digest of soyabean

Directions

Suspend 44.77 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml R-water/purified/distilled water with 10 ml polysorbate 80. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Adjust the pH of the medium, using freshly prepared buffer solution as recommended by the British Pharmacopoeia for the antibiotic assayed.

Principle And Interpretation

Antibiotic Assay Medium B is prepared according to British Pharmacopoeia (1) and is used for the microbiological assay of Colistimethate sodium using *Bordetella bronchiseptica* ATCC 4617 and *Escherichia coli* ATCC 10536.

Combination of tryptone and soya peptone provides nitrogenous and carbonaceous compounds, long chain amino acids and other essential nutrients for the growth of test organisms. Glucose monohydrate provides fermentable source of carbon, and enhances the growth of test organism. Phosphate in the medium enhances buffering action and sodium chloride maintains osmotic equilibrium.

Freshly prepared plates should be used for antibiotic assays. Test organisms are inoculated in sterile seed agar 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.

Type of specimen

Pharmaceutical samples

Specimen Collection and Handling

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (1).

After use, contaminated materials must be sterilized by autoclaving before discarding.

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. Freshly prepared medium plates must be used or it may result in erroneous results.
**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**
Cream to yellow homogeneous free flowing powder

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

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

**pH**
7.20-7.40

**Cultural Response**
Cultural characteristics observed after an incubation at 35-39°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><em>Bordetella bronchiseptica</em> ATCC 4617</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td>Colistimethate sodium</td>
</tr>
<tr>
<td><em>Escherichia coli ATCC 10536</em></td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
<td>Colistimethate sodium</td>
</tr>
</tbody>
</table>

**Storage and Shelf Life**
Store between 10-30°C in a tightly closed container and use freshly prepared medium. 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

**Reference**

1. British Pharmacopoeia, 2016, British Pharmacopoeia Commission

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