Antibiotic Assay Medium A with pH 7.9

Intended use
Antibiotic Assay Medium A with pH 7.9 is used for microbiological assay of antibiotics.

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>Tryptone$</td>
<td>4.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>3.000</td>
</tr>
<tr>
<td>HM Peptone B#</td>
<td>1.500</td>
</tr>
<tr>
<td>Glucose monohydrate</td>
<td>1.000</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>pH after sterilization (at 25°C)</td>
<td>7.9±0.1</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters
$-$ Equivalent to Pancreatic digest of casein
#- Equivalent to Beef extract

Directions
Suspend 30.45 grams (the equivalent weight of dehydrated medium per litre) 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 Gentamicin sulphate, Kanamycin monosulphate, Kanamycin acid sulphate, Neomycin sulphate, Netilmicin sulphate, Spiramycin, Streptomycin sulphate, Tylosin, Tylosin tartarate, Vancomycin hydrochloride.

Principle And Interpretation
Antibiotic Assay media are used in the performance of antibiotic assays. Grove and Randall have elucidated those antibiotic assays and media in their comprehensive treatise on antibiotic assays (2). Schmidt and Moyer have reported the use of antibiotic assay medium for the liquid formulation used in the performance of antibiotic assay (5). This medium is recommended by BP (1) and FDA (6).

Nutrients and growth factors are supplied by the ingredients like peptone, tryptone, yeast extract and HM peptone B.
Dextrose provides the carbon and energy source. Agar provides excellent medium for antibiotic diffusion and gives well-defined zones of inhibition. Higher pH provides the optimal conditions for activity of antibiotic and also supports the growth of the test organisms.

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.

Type of specimen
Pharmaceutical preparations

Specimen Collection and Handling
For pharmaceutical samples follow appropriate techniques for handling specimens as per established 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 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 coloured homogeneous free flowing powder

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

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

**pH**  
7.80-8.00

**Growth Promotion Test**  
As per British Pharmacopoeia

**Cultural Response**  
M004B: Cultural characteristics observed after an incubation at specified temperature for 18-24 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
<th>Antibiotics assayed &amp; incubation temp.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cultural Response</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Micrococcus luteus ATCC 9341</em></td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>≥70%</td>
<td>Tylosin, Tylosin tartarate (adjust the pH to 8.0±0.1) 32-35°C</td>
</tr>
<tr>
<td><em>Staphylococcus aureus ATCC 6538p (00195</em>)*</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>≥70%</td>
<td>Kanamycin mono-sulphate / 30-37°C, Kanamycin acid sulphate/35-39°C, Netilmycin sulphate/32-35°C</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis ATCC 12228 (00036</em>)*</td>
<td>50-100</td>
<td>luxuriant</td>
<td>≥70%</td>
<td>Gentamicin sulphate/35-39°C</td>
</tr>
<tr>
<td><em>Bacillus pumilis NCTC 8241</em></td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>≥70%</td>
<td>Gentamicin sulphate/35-39°C, Neomycin sulphate/30-37°C</td>
</tr>
<tr>
<td><em>Bacillus subtilis subsp. spizizenii ATCC 6633 (00003</em>)*</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>≥70%</td>
<td>Kanamycin mono sulphate /30-37°C, Kanamycin acid sulphate/35-39°C, Neomycin sulphate/30-37°C, Spiramycin / 30-32°C, Streptomycin sulphate /30-37°C, C Vancomycin hydrochloride (adjust the pH to 8.0±0.1) / 37-39°C</td>
</tr>
<tr>
<td><em>Bacillus subtilis NCTC 8236</em></td>
<td>50-100</td>
<td>luxuriant</td>
<td>≥70%</td>
<td>Streptomycin sulphate/30-37°C</td>
</tr>
</tbody>
</table>

* Corresponding WDCM numbers
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 (3,4).

Reference
5. Schmidt and Moyer, 1944; J. Bact, 47:19