Medium 21. Pseudomonas Agar Medium for Detection of Pyocyanin

Pseudomonas Agar for detection of Pyocyanin is recommended for the detection of pyocyanin production by Pseudomonas species in accordance with Indian Pharmacopoeia, 2007.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic digest of gelatin</td>
<td>20.000</td>
</tr>
<tr>
<td>Anhydrous potassium sulphate</td>
<td>10.000</td>
</tr>
<tr>
<td>Anhydrous magnesium chloride</td>
<td>1.400</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>pH after sterilization (at 25°C)</td>
<td>7.2±0.2</td>
</tr>
</tbody>
</table>

**Directions**

Suspend 46.4 grams in 1000 ml purified / distilled water containing 10 ml glycerin. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes or as per validated cycle.

**Principle And Interpretation**

Pseudomonas Agar is based on the formulation described by King et al (1) and as recommended in Indian Pharmacopoeia (2) for detecting pyocyanin, a water soluble pigment by Pseudomonas species from pharmaceutical preparation and clinical specimens such as stools, wounds, and urine. (3). Pseudomonas species are commonly isolated pathogen and is the significant causative agent of nosocomial, skin and burn infections. Pseudomonas strains are reported to produce phenazine pigments like Pyocyanin- blue green redox-active secondary metabolite pigment, pyorubin-rust brown pigment, -oxyphenzine- a breakdown product of Pyocyanin, pyoverdin-a water soluble yellow green pigments also known as fluorescein. Pyocyanin is readily recovered in large quantities in sputum from patients with cystic fibrosis, an infection caused by Pseudomonas (4,5). This medium enhances the formation of Pyocyanin and/or pyorubin and reduces that of fluorescein.

Pancreatic digest of gelatin provides essential nutrients for growth of Pseudomonas, while glycerol provides carbon and energy to the cell. The pyocyanin pigment diffuses from the colonies of Pseudomonas into the agar and shows blue colouration. Potassium sulphate and magnesium chloride enhances the pyocyanin production and suppresses the fluorescein production. Low content of phosphorous in the medium also aids in inhibiting the production of fluorescein. Some Pseudomonas strains produce small amounts of fluorescein resulting in a blue-green colouration.

Some of Pseudomonas aeruginosa that may fail to produce Pyocyanin are not detected in this medium. Production of other pigments may mask the presence of Pyocyanin.

**Quality Control**

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

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

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

**pH**
7.00-7.40

**Cultural Response**

---

Please refer disclaimer Overleaf.
Growth Promotion is carried out in accordance with the harmonized method of IP. Cultural response was observed after an incubation at 33-37°C for not less than 3 days. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

### Cultural Response

#### Test for Pseudomonas aeruginosa

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Observed Lot value (CFU)</th>
<th>Recovery</th>
<th>Characteristic colonial morphology</th>
<th>Fluorescence in UV light</th>
<th>Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas aeruginosa ATCC 9027</td>
<td>50 -100</td>
<td>35 -100</td>
<td>&gt;=70 %</td>
<td>Generally greenish</td>
<td>positive</td>
<td>positive</td>
</tr>
</tbody>
</table>

#### Additional Microbiological Testing

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Observed Lot value (CFU)</th>
<th>Recovery</th>
<th>Characteristic colonial morphology</th>
<th>Fluorescence in UV light</th>
<th>Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas aeruginosa ATCC 27853</td>
<td>50 -100</td>
<td>35 -100</td>
<td>&gt;=70 %</td>
<td>Generally greenish</td>
<td>positive</td>
<td>positive</td>
</tr>
</tbody>
</table>

### Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

### Reference

2. Indian Pharmacopoeia, 2007, Govt. of India, Ministry of Health and Family Welfare, New Delhi, India.

Revision : 2 / 2015

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.