Pseudomonas Agar Medium for Detection of Pyocyanin

Pseudomonas Agar Medium for detection of Pyocyanin is recommended for the detection of pyocyanin production by Pseudomonas species 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 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>
</tbody>
</table>

pH after sterilization (at 25°C) 7.2±0.2

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

**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 by U.S. Pharmacopoeia (2) for detecting pyocyanin, a water soluble pigment by Pseudomonas species from clinical specimens such as stools, wounds, and urine. (3). It is also recommended for microbial limit tests for pharmaceutical and other biological preparations by USP. 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 casein 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.

Strains 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**
Growth Promotion is carried out in accordance with the harmonized method of USP. 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**

<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>Oxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Test for Pseudomonas aeruginosa</strong></td>
<td>50 -100</td>
<td>35 -100</td>
<td>&gt;=70 %</td>
<td>Generally greenish</td>
<td>Blue</td>
<td>positive</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>ATCC 9027</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Additional Microbiological Testing</strong></td>
<td>50 -100</td>
<td>35 -100</td>
<td>&gt;=70 %</td>
<td>Generally greenish</td>
<td>blue</td>
<td>positive</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>ATCC 27853</td>
<td></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 the prepared medium at 2 - 8°C. Use before expiry date on the label.

**Reference**


Revision : 2 / 2015

**Disclaimer :**

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