Antimycotic Sensitivity Test Agar

Antimycotic Sensitivity Test Agar is used for testing antimycotic sensitivity by diffusion method using antimycotic sensitivity discs.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein enzymic hydrolysate</td>
<td>19.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>10.000</td>
</tr>
<tr>
<td>Glucose</td>
<td>20.000</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>10.000</td>
</tr>
<tr>
<td>Disodium phosphate</td>
<td>1.000</td>
</tr>
<tr>
<td>Agar</td>
<td>25.000</td>
</tr>
<tr>
<td>Final pH ( at 25°C)</td>
<td>6.6±0.2</td>
</tr>
</tbody>
</table>

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

**Directions**

Suspend 85.0 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well before pouring into sterile petri plates.

**Principle And Interpretation**

**Technique:**

1. **Preparation of Inoculum:**
   1. Inoculum is prepared by picking five distinct colonies of approximately 1mm from 24 hours old culture grown on Sabouraud Dextrose Agar (M063) and incubated at 35 ± 2°C. Colonies are suspended in 5ml of sterile 0.85% Saline.
   2. Vortex the resulting suspension and adjust the turbidity to yield 1 x 106 - 5 x 106 cells /ml (i.e. 0.5 McFarland standard).

2. **Test Procedure:**
   1. Prepare plates with Antimycotic sensitivity Test Agar for carrying out susceptibility of antifungal discs. The medium in the plates should be sterile and have a depth of about 4 mm.
   2. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum (turbidity so adjusted, as to obtain semi confluent growth on the petri plate) and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
   3. Apply the discs using aseptic technique. Deposit the discs with centers at least 24 mm apart.
   4. Invert the plates and place in an incubator set to 35 ± 2°C within 15 minutes after the discs are applied.
   5. Examine each plate after 20 - 24 hours of incubation. If plate was satisfactorily streaked the resulting zones of inhibition will be uniformly circular and there will be a semi-confluent lawn of growth. Read at 48 hours only when insufficient growth is observed after 24 hours incubation.

Antimycotic Sensitivity Test Agar is recommended for testing the antimycotic activity by disc diffusion method (Sensitivity disc or MIC testing). The Clinical and Laboratory Standards Institute (CLSI) subcommittee on Antifungal Susceptibility Testing (1) has established interpretative break point for three drugs and Candida spp. The M44-A document (approved standard) became available in 2004. (2) Espinel-Ingroff (3)suggested that easier test modification to CLSI methods are desirable. Several workers have used agar diffusion method as an alternative approch to the CLSI methods. Stiller et al (4) observed a good correlation between MICs and growth inhibition zones for 5-FC. Pfaller et al (5) found a fluconazole disk test to be comparable to the MIC test. Barry and Brown (6) demonstrated good correlation between fluconazole disk test and MIC determined by
either broth dilution or E Test. Espinel et al (7) and Pfaffer et al (8) evaluated E Test and suggested that it correlates well with CLSI reference methods. However this agreement was species and medium dependent and they suggested the need for further optimisation of medium formulation.

The medium contains casein enzymic hydrolysate and yeast extract which provide all essential growth nutrients like amino acids, vitamins, trace elements etc. Glucose serves as energy source. Disodium phosphate buffers the medium well.

**Quality Control**

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

**Gelling**
Firm, comparable with 2.5% Agar gel.

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

**Reaction**
Reaction of 8.5% w/v aqueous solution at 25°C. pH : 6.6±0.2

**pH**
6.40-6.80

**Cultural Response**
M1336: Average diameter of zone of inhibition observed after an incubation at 25-30°C for 48-72 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Growth</th>
<th>Zone of inhibition (Amphotericin B 50mcg)</th>
<th>Zone of inhibition (Nystatin 50mcg)</th>
<th>Zone of inhibition (Miconazole 50mcg)</th>
<th>Zone of inhibition (Miconazole 30mcg)</th>
<th>Zone of inhibition (Ketoconazole 50mcg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida albicans ATCC 90028</em></td>
<td>Luxuriant</td>
<td>12 - 15mm</td>
<td>19 - 23mm</td>
<td>26 - 32mm</td>
<td>22-26mm</td>
<td>30 - 37mm</td>
</tr>
<tr>
<td><em>Candida parapsillosis ATCC 11-18mm 22019</em></td>
<td>13 - 17mm</td>
<td>19 - 23mm</td>
<td>23 - 29mm</td>
<td>13 - 17mm</td>
<td>27 - 33mm</td>
<td></td>
</tr>
</tbody>
</table>

**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**
4. Zone Diameter Interpretive Standards, Corresponding Minimal Inhibitory Concentration (MIC) Interpretive Breakpoints, and Quality Control Limits for Antifungal Disk Diffusion Susceptibility Testing Of Yeasts, Third International Supplement CLSI document - M44-S3.

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