Antimicrobial Inhibitor Test Agar pH 7.2, Granulated

Antimicrobial Inhibitor Test Agar pH 7.2, granulated is recommended for residual analysis of antimicrobial components in meat and organ samples, using *Bacillus subtilis* (ATCC 6633) as test organism.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>7.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Trisodium phosphate, 12H₂O</td>
<td>0.800</td>
</tr>
<tr>
<td>Agar</td>
<td>13.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.2</td>
</tr>
</tbody>
</table>

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

**Directions**

Suspend 25.34 grams (the equivalent weight of dehydrated medium per litre) 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. Cool to 45-50°C. Mix 1 ml of *Bacillus subtilis* spore suspension per liter of sterile and cooled (45-50°C) medium. Add 50µg of trimethoprim per litre of medium. Mix thoroughly and pour into sterile Petri plates.

**Principle And Interpretation**

In addition to washing, treatments with antimicrobial compounds such as chlorine and organic acids are used for sanitization. Various concentrations and the degree of effectiveness of the concentrations of these antimicrobial compounds have been reported. Antimicrobial Inhibitor Test Agar pH 7.2 is recommended for residual analysis of antimicrobial components in meat and organ samples, using *Bacillus subtilis* (ATCC 6633) as test organism by agar diffusion procedure and EEC Four-Plate-Test.

Agar Diffusion procedure: Small slices of the meat sample are placed on the seeded Test agar plates and incubated. Antimicrobial components, if present in the sample, diffuse into the medium and inhibit the growth of test organisms causing growth free inhibition zone. This test is repeated with all the three Antimicrobial Inhibitor Test Agar with three different pH i.e. GM1631/M1631 (pH 6.0), GM1601/M1601 (pH 7.2) and GM1632/M1632 (pH 8.0). Different antibiotic to be analysed such as penicillin, streptomycin and sulphonamide have different pH range for optimal activity for example penicillin G is active optimally at pH 6.0 and streptomycin at pH 8.0 and sulphonamide at pH 7.2. Trimethoprim (50µg) is added to increase the sensitivity of test agar for residual sulphonamide from meat/organ samples (1).

For EEC Four-Plate-Test method: Molten and cooled antimicrobial inhibitor test agar is seeded with test organisms, *Bacillus subtilis* spores suspension (2), mixed well and dispensed into Petri plates. In one half of the seeded plate, aseptically clump small piece (2-8mm) of meat or organ sample at proper distance and in second half of the plate, place test discs with standard antibiotic to be analyzed, as control. Use disc with 0.5µg sulphonamide as standard.

Incubate the plates for 18-24 hours at 30°C for *Bacillus subtilis*. After incubation measure the zone of inhibition. The inhibition zone between tissue section edge and growth limit of test organism is measured. The zone of at least 2 mm is regarded as positive and less than 2mm (12mm) is considered doubtful. The standard disc should display minimum 6 mm zone of inhibition.

Antimicrobial Inhibitor Test Agar pH 7.2 has peptone which serve as source for carbon, nitrogen and growth factors for the growth of organisms. Sodium chloride helps to maintain osmotic balance in the medium and phosphate salt offers buffering capacity to medium.

**Quality Control**

**Appearance**

Please refer disclaimer Overleaf.
Cream to yellow coloured granular media

**Gelling**
Firm, comparable with 1.3% Agar gel.

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

**Reaction**
Reaction of 2.53% w/v aqueous solution at 25°C. pH : 7.2

**pH**
7.20

**Cultural Response**
Cultural response and zone of inhibition was observed using *B. subtilis* after an incubation at 30ºC for 18-24 hours

| Organism            | Growth           | Inhibition zones with Gentamicin (10mcg) | Inhibition zones with Gentamicin (30mcg) | Inhibition zones with Penicillin (0.01IU) | Inhibition zones with Penicillin (10 IU) | Inhibition zones with Streptomycin (0.5mcg) | Inhibition zones with Streptomycin (10mcg) | Inhibition zones with Sulphadimine (0.5mcg) | Inhibition zones with Sulphadimine (10mcg) |
|---------------------|------------------|------------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|
| *Bacillus subtilis* | good-luxuriant   | 30-35mm                                   | 35-40mm                                  | 12-20mm                                  | 25-35mm                                  | 15-20mm                                  | 25-30mm                                  | 12-20mm                                  | 30-35mm                                  |

**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. Ferrini, A. M.; Mannoni, V., Aaurdi P. Combined plate microbal assay (CPMA). Food additives and Contaminants, 23(1);16-24. 2006

Revision : 00 / 2014

**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.