M-Azide Broth Base

M-Azide Broth Base is a selective medium used for cultivation and enumeration of Enterococci from water samples using membrane filter technique.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptose</td>
<td>40.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>10.000</td>
</tr>
<tr>
<td>Dextrose</td>
<td>2.000</td>
</tr>
<tr>
<td>Saccharose</td>
<td>100.000</td>
</tr>
<tr>
<td>Dipotassium phosphate</td>
<td>4.000</td>
</tr>
<tr>
<td>Sodium azide</td>
<td>0.400</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.1±0.2</td>
</tr>
</tbody>
</table>

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

**Directions**

Suspend 15.64 grams in 100 ml distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C and aseptically add 1 ml of 1% 2, 3, 5 Triphenyl Tetrazolium Chloride (TTC, FD057). Mix well before dispensing.

Caution: Sodium azide has a tendency to form explosive metal azides with plumbing materials. It is advisable to use enough water to flush off the disposables.

**Principle And Interpretation**

Enterococci may be considered an essential part of the autochthonous microflora of humans and animals. Because of its wide distribution, Enterococci can also occur in different food commodities, especially those of animal origin (1, 2). The most important feature of this genus is their high level of endemic antibiotic resistance. In bodies of water, the acceptable level of contamination is very low. In 2004, *Enterococcus* species took the place of fecal coliform as the new federal standard for water quality at public beaches. It is believed to provide a higher correlation than fecal coliforms with many of the human pathogens often found in sewage (3).

M-Azide Broth was formulated by Slanetz, Bent and Bartely (4) and is especially recommended for the enumeration of Enterococci from water samples and other specimens using membrane filter technique. In this technique, a measured volume of the water sample is filtered through a membrane with a pore size small enough to retain the indicator bacteria to be counted. The membrane is then aseptically placed and incubated on a selective indicator medium (or sterile absorbent cotton pads saturated with the selective medium), so that the indicator bacteria grow into colonies on its upper surface (5).

Tryptose, yeast extract provide essential growth nutrients. Dextrose and saccharose are the fermentable carbohydrates. Sodium azide is used as selective agent, which inhibits gram-negative bacteria. Mallmann et al (6) reported that sodium azide exerts bacteriostatic effect on gram-negative bacteria allowing unrestricted growth of gram-positive cocci, particularly Enterococci. TTC imparts pink to red colour to the colonies.

For membrane filter technique (7), 2.2 ml medium is added per absorbent pad. Using this medium, Slanetz et al observed better recovery of pure cultures of *Enterococcus faecalis* by membrane filter technique than MPN technique.

**Quality Control**

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

**Colour and Clarity of prepared medium**
Light yellow coloured clear solution without any precipitate

**Reaction**

Please refer disclaimer Overleaf.
Reaction of 15.64% w/v aqueous solution at 25°C. pH : 7.1±0.2

**pH**
6.90-7.30

**Cultural Response**
M1119: Cultural characteristics observed after an incubation at 35-37°C for 48 hours with added 1% 2,3,5 Triphenyl Tetrazolium Chloride (TTC, FD057).

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Colour of colony (on membrane filter)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> ATCC 25922</td>
<td>&gt;=10³</td>
<td>inhibited</td>
<td>-</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> ATCC 50-100</td>
<td>good-luxuriant</td>
<td>pink to red</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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