Tetrathionate Broth Medium

**Tetrathionate Broth Medium** is recommended as a selective enrichment broth for isolation of *Salmonella Typhi* and other *Salmonellae* from clinical specimens and biologicals as specified in Indian Pharmacopoeia, 1996

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat extract B #</td>
<td>0.900</td>
</tr>
<tr>
<td>Peptone</td>
<td>4.500</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>1.800</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>4.500</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>25.000</td>
</tr>
<tr>
<td>Sodium thiosulphate</td>
<td>40.700</td>
</tr>
</tbody>
</table>

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

# Equivalent to Beef extract

**Directions**

Suspend 77.40 grams in 980 ml purified/distilled water. Heat just to boiling. Cool below 45-50°C and aseptically add 20 ml iodine solution (6 grams of iodine and 5 grams of potassium iodide in 20 ml distilled water). Mix well and dispense in 10 ml quantities in sterile tubes.

This complete medium should be used on the day of preparation. Do not heat after the addition of iodine solution. Use the medium immediately after addition of iodine.

**Note**: Due to presence of calcium carbonate, the prepared medium forms opalescent solution with a white precipitate.

**Principle And Interpretation**

Tetrathionate Broth Medium was originally described by Mueller (1) and found that the medium selectively inhibit coliforms and permit unrestricted growth of enteric pathogens. The medium is now formulated according to Indian Pharmacopoeia (2), Compendium of Microbiological Examination of Foods (3) and Standard Methods for the Examination of Water and Wastewater (4) specify this medium as enrichment medium for *Salmonella* species. *Salmonella* is the common causative agent of mild gastroenteritis to typhoid. It is common contaminant in food and other biological products. This medium supports the rejuvenation of *Salmonella* cells injured by food processing which are incapable of forming colonies on plate, but on injection can cause infection.

The selectivity depends on the ability of thiosulphate and tetrathionate (formed by addition of Iodine and Potassium iodide) in combination to suppress commensal coliform organisms (5,6). The microorganism harboring tetrathionate reductase flourish in this broth. Sodium thiosulphates are inactivators of halogens and can minimize its toxicity in the testing sample, if any during microbial limit tests. Yeast extract, meat extract B and peptone provides essential nutrients, growth factors and vitamins in this medium. Calcium carbonate neutralizes the acidic tetrathionate decomposition products. Sodium chloride maintains osmotic balance.

For further confirmation, streak the enriched cultures after incubation, on the plates of Brilliant Green Agar (MM016), MacConkey Agar (MM081), Bismuth Sulphite Agar (MM027).

**Quality Control**

**Appearance**

Cream to yellow homogeneous free flowing powder

**Colour and Clarity of prepared medium**

Complete medium with added brilliant green and iodine solution - Light green opalescent with white precipitate, on standing the precipitate settles down.

**Growth Promotion Test**

As per Indian Pharmacopoeia.
Cultural Response
Cultural characteristics observed with added brilliant green and iodine solution when sub cultured on Xylose Lysine Deoxycholate Agar (MM031) after enrichment in Tetrathionate medium, after an incubation at 35-37°C for 18-72 hours.

Cultural Response

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Observed Lot value (CFU)</th>
<th>Recovery</th>
<th>Colour of Colony</th>
<th>Incubation temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella Typhimurium ATCC 14028</td>
<td>50 -100</td>
<td>luxuriant</td>
<td>25 -100</td>
<td>&gt;=50 %</td>
<td>red with black centres</td>
<td>18 -72 hrs</td>
</tr>
<tr>
<td>Salmonella Abyon NCTC 6017</td>
<td>50 -100</td>
<td>good-luxuriant</td>
<td>25 -100</td>
<td>&gt;=50 %</td>
<td>red with black centres</td>
<td>18 -72 hrs</td>
</tr>
</tbody>
</table>

Storage and Shelf Life
Store below 30°C in tightly closed container. Use before expiry date on the label.

Reference
2. The Indian Pharmacopoeia (1996), Vol. II.

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