Fluid Tetrathionate Medium

Fluid Tetrathionate Medium in combination with added iodine and brilliant green is recommended as an enrichment broth for isolation of Salmonellae from specimens suspected to be contaminated with Salmonellae 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 casein</td>
<td>2.500</td>
</tr>
<tr>
<td>Peptic digest of animal tissue</td>
<td>2.500</td>
</tr>
<tr>
<td>Bile salts</td>
<td>1.000</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>10.000</td>
</tr>
<tr>
<td>Sodium thiosulphate</td>
<td>30.000</td>
</tr>
</tbody>
</table>

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

**Directions**

Suspend 46 grams in 970 ml purified/distilled water and heat just to boiling. DO NOT AUTOCLAVE. Cool to 45-50°C. On the day of use add 20 ml iodine solution (iodine - 6 grams and potassium iodide - 5 grams in 20 ml distilled water) and 10 ml of 0.1% brilliant green solution. Mix well and dispense in 10 ml quantities.

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**

Fluid Tetrathionate 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 FDA (2), United States Pharmacopoeia, 2009 (3), Compendium of Microbiological Examination of Foods (4) and Standard Methods for the Examination of Water and Wastewater (5) which 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. This medium is recommended by USP for microbial limit tests for pharmaceutical preparations.

Bile salts inhibit gram-positive microorganisms. 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 (6,7). The microorganism harbouring tetrathionate reductase flourish in this broth. Sodium thiosulphate forms the substrate for enzyme thiosulphate reductase. Sodium thiosulphates are also inactivators of halogens and can minimize its toxicity in the testing sample, if any during microbial limit tests. Pancreatic digest of casein and peptic digest of animal tissues supplies essential nutrients 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 (MU016), MacConkey Agar (MU081), Bismuth Sulphite Agar (MU027).

**Quality Control**

**Appearance**

White to cream homogeneous free flowing powder

**Colour and Clarity of prepared medium**

Complete medium with added brilliant green and iodine solution - Light green coloured opalescent solution with white precipitate, on standing the precipitate settles down.
Growth Promotion Test
As per United States Pharmacopoeia.

Cultural Response
Cultural characteristics observed with added brilliant green and iodine solution, after an incubation at 35-37°C for 18-24 hours, when sub cultured on MacConkey Agar (MU081) after enrichment in Tetrathionate medium.

Cultural Response

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Recovery</th>
<th>Colour of colony</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella Abony NCTC 6017</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>colourless</td>
</tr>
<tr>
<td>Salmonella Typhimurium ATCC 14028</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>colourless</td>
</tr>
<tr>
<td>Salmonella Typhi ATCC 6539</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>colourless</td>
</tr>
<tr>
<td>Escherichia coli ATCC 8739</td>
<td>50-100</td>
<td>little or no increase in numbers</td>
<td>white to pink with bile precipitate</td>
</tr>
</tbody>
</table>

Storage and Shelf Life
Store below 30°C and the base broth at 2 - 8°C. Use before expiry date on the label.

Reference

Revision : 2 / 2014

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