Rappaport Vassiliadis Salmonella Enrichment Broth

**Intended use**

Rappaport Vassiliadis Salmonella Enrichment Broth is recommended for selective enrichment of *Salmonella* species from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP.

**Composition**

- **Ingredients**
  - Soya peptone: 4.500 Gms / Litre
  - Sodium chloride: 8.000
  - Dipotassium phosphate: 0.400
  - Potassium dihydrogen phosphate: 0.600
  - Magnesium chloride, hexahydrate: 29.000
  - Malachite green: 0.036
  - pH after sterilization (at 25°C): 5.2±0.2

**Directions**

Suspend 27.11 grams of dehydrated medium (the equivalent weight of dehydrated medium per litre) in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Dispense as desired into tubes and sterilize by autoclaving at 115°C as per validated cycle.

**Principle And Interpretation**

Rappaport Vassiliadis Salmonella Enrichment Medium is designed according to the revised formulation by Van Schothorst et al (1) and is recommended for the selective enrichment of Salmonellae from pharmaceutical products. This medium can also be used in direct enrichment of samples containing low inoculum. Present medium is a modification of the Rappaport Vassiliadis Enrichment Broth described by Van Schothorst and Renauld (2). It is prepared in accordance with the harmonized methodology of USP/EP/BP/JP/IP (3,4,5,6,11) has been found to be superior to other *Salmonella* selective medias. Addition of magnesium chloride to the medium was reported by Peterz et al (7). *Salmonella* species can be isolated from human faeces without pre-enrichment by using this medium.

*Salmonella* generally survive at little high osmotic pressure, grow at slightly low pH and are resistant to malachite green compared to other bacteria. These characteristics are exploited in this medium for selective enrichment of Salmonella. Magnesium chloride present in the medium raises the osmotic pressure. Natural sugars of soya peptone provide essential growth nutrients and enhance the growth of *Salmonella* (8). Phosphate buffers the medium to maintain constant pH. Sodium chloride maintains the osmotic balance. Malachite green inhibits many gram-positive bacteria, while selectively enriches *Salmonella*.

The relatively lower concentration of nutrition, also aids selective enrichment of *Salmonella*. This medium was reported to be superior to *Salmonella* selective medium like Tetrathionate Broth and Selenite enrichment broth and to Tetrathionate-Brilliant Green Broth for the detection of Salmonellae in milk samples. The enriched culture of Rappaport Vassiliadis Salmonella Enrichment Broth (MH1491) can be further subcultured and isolated on Xylose Lysine Deoxycholate Agar (MH031).

**Type of specimen**

Pharmaceutical samples

**Specimen Collection and Handling**

For pharmaceutical samples follow appropriate techniques for handling specimens as per established guidelines (3,4,5,6,11). After use, contaminated materials must be sterilized by autoclaving before discarding.

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Please refer disclaimer Overleaf.
Warning and Precautions:
Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations:
Overheating may destroy the selectivity of medium.

Performance and Evaluation
Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control
Appearance
Light yellow to light blue homogeneous free flowing powder
Colour and Clarity of prepared medium
Greenish blue coloured clear to slightly opalescent solution with a slight precipitate in tubes.
PH
5.00-5.40
Cultural Response
Growth Promotion is carried out in accordance with harmonized method of USP/BP/EP/JP/IP. Cultural response was observed after an incubation at 30-35°C for specified time. Recovery is carried out using Xylose Lysine Deoxycholate Agar (MH031), after enrichment in Rappaport Vassiliadis Salmonella Enrichment Broth.
Growth promoting properties
Clearly visible growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating <=100 cfu (at 30-35°C for <=18 hours).
Inhibitory properties
No growth of the test microorganism occurs for the specified temperature for not less than longest period of time specified inoculating >=100 cfu (at 30-35°C for >= 24 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><strong>Growth promoting</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Salmonella Typhimurium</em> ATCC 14028</td>
<td>50 -100</td>
<td>luxuriant</td>
<td>&gt;=35</td>
<td>&gt;=70 %</td>
<td>red with black</td>
<td>&lt;=18 hrs</td>
</tr>
<tr>
<td><em>Salmonella Abony</em> NCTC 6017 (00029*)</td>
<td>50 -100</td>
<td>luxuriant</td>
<td>&gt;=35</td>
<td>&gt;=70 %</td>
<td>red with black</td>
<td>&lt;=18 hrs</td>
</tr>
<tr>
<td><strong>Inhibitory</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 6538</td>
<td>&gt;=10³</td>
<td>inhibited</td>
<td>0</td>
<td>0 %</td>
<td>yellow</td>
<td>&gt;=24 hrs</td>
</tr>
<tr>
<td><strong>Additional Microbiological testing</strong></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 25922 (00013*)</td>
<td>50 -100</td>
<td>none-poor</td>
<td>0 -10</td>
<td>0 -10 %</td>
<td>yellow</td>
<td>18 -24 hrs</td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 8739 50 -100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(00012*)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Salmonella Enteritidis</em> ATCC 50 -100</td>
<td></td>
<td>luxuriant</td>
<td>&gt;=35</td>
<td>&gt;=70 %</td>
<td>red with black</td>
<td>18 -24 hrs</td>
</tr>
<tr>
<td>13076 (00030*)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><em>Salmonella Paratyphi B</em> ATCC 8759</td>
<td>50 -100</td>
<td>luxuriant</td>
<td>&gt;=35</td>
<td>&gt;=70 %</td>
<td>red with black</td>
<td>18 -24 hrs</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 25923</td>
<td>&gt;=10³</td>
<td>inhibited</td>
<td>0</td>
<td>0 %</td>
<td></td>
<td>&gt;=24 hrs</td>
</tr>
</tbody>
</table>
### Technical Data

<table>
<thead>
<tr>
<th>Strain</th>
<th>Concentration</th>
<th>Result</th>
<th>Time</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas aeruginosa</em> ATCC 9027</td>
<td>&gt;=10^3</td>
<td>inhibited</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> ATCC 27853</td>
<td>&gt;=10^3</td>
<td>inhibited</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> ATCC 29212</td>
<td>&gt;=10^1</td>
<td>inhibited</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td><em>E.coli</em> + <em>S.Typhimurium</em> (mixed culture)</td>
<td>50 -100</td>
<td>luxuriant</td>
<td>&gt;=35</td>
<td>&gt;=70%</td>
</tr>
<tr>
<td><em>Salmonella Typhimurium</em> ATCC 14028</td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Key: *Corresponding WDCM numbers.

### Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Use before expiry date on the label.

Product performance is best if used within stated expiry period.

### Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical samples should be decontaminated and disposed of in accordance with current laboratory techniques (9,10).

### Reference

11. Indian Pharmacopoeia, 2017, Govt. of India, Ministry of Health and Family Welfare, New Delhi

Disclaimer:

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