Rappaport Vasiliadis Salmonella Enrichment Broth (As per IP)  

**Intended use**
For selective enrichment of *Salmonella* species from pharmaceutical & clinical sample in accordance Indian pharmacopoeia

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soya peptone</td>
<td>4.500</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>8.000</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate</td>
<td>0.400</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>0.600</td>
</tr>
<tr>
<td>Magnesium chloride, hexahydrate</td>
<td>29.000</td>
</tr>
<tr>
<td>Malachite green</td>
<td>0.036</td>
</tr>
</tbody>
</table>

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

**Directions**
Label the ready to use LQ104I bottle. Inoculate the sample and Incubate at specified temperature and time.

**Principle And Interpretation**

Rappaport Vassiliadis Salmonella Enrichment Medium is designed according to the revised formulation by Van Schothorst et al (10) 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 (11). It is prepared as per the formulation in Indian Pharmacopoeia (3) and in accordance with harmonized methodology of USP/EP/BP/JP (9,1,2,5). Addition of magnesium chloride to the medium has been found to be superior to other *Salmonella* selective media by Peterz et al (8). *Salmonella* species can be isolated from human faeces without pre-enrichment by using this medium.

*Salmonella* species 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* (4). 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 (MM1491) can be further subcultured and isolated on Xylose Lysine Deoxycholate Agar (MM031).

**Type of specimen**
Pharmaceutical samples, Clinical samples: faecal dilution

**Specimen Collection and Handling**
For pharmaceutical samples follow appropriate techniques for handling specimens as per established guidelines (9,1,2,5,3). After use, contaminated materials must be sterilized by autoclaving before discarding.
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
Due to variable nutritional requirements, some strains may show poor growth on this 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
Sterile clear Rappaport Vassiliadis Salmonella Enrichment Broth in bottles.

Colour
Bluish green coloured solution.

Quantity of medium
10 ml of medium in bottles.

Reaction
5.00-5.40

Sterilization Method
Sterilized by autoclaving at 115 °C as per validated cycle

Sterility Assurance Level
Sterility assurance level of media was validated against *B.subtilis* Spore strips. The spore strips exposed at 115°C and unexposed strips were inoculated separately in 100ml Soyabean Casein Digest Medium and incubated at 35°C for 7 days.

Exposed spore strips
No growth observed

Unexposed spore strips
Luxuriant growth observed

Sterility test
Passes release criteria

Cultural Response
Cultural characteristics observed after incubation at 30-35°C for 18-24 hours. Recovery is carried out using XLD Agar (MM031).

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 least 100 cfu) (at 30-35°C for >=24 hours).

<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>Growth promoting</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Salmonella Typhimurium ATCC 14028 (00031</em>)*</td>
<td>50 -100</td>
<td>luxuriant</td>
<td>&gt;=35</td>
<td>&gt;=70 %</td>
<td>red with black centers</td>
<td>&lt;=18 hrs</td>
</tr>
<tr>
<td><em>Salmonella Abony NCTC 6017 (00029</em>)*</td>
<td>50 -100</td>
<td>luxuriant</td>
<td>&gt;=35</td>
<td>&gt;=70 %</td>
<td>red with black centers</td>
<td>&lt;=18 hrs</td>
</tr>
<tr>
<td>Inhibitory</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> subsp. <em>aureus ATCC 6538 (00032</em>)*</td>
<td>&gt;=10³</td>
<td>inhibited</td>
<td>0</td>
<td>0 %</td>
<td></td>
<td>&gt;=24 hrs</td>
</tr>
</tbody>
</table>

Please refer disclaimer Overleaf.
**Additional Microbiological testing**

<table>
<thead>
<tr>
<th>Strain</th>
<th>MPN (CFU/g)</th>
<th>Growth</th>
<th>Color</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> ATCC 25922</td>
<td>50 - 100</td>
<td>none-poor</td>
<td>yellow</td>
<td>18 - 24 hrs</td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 8739</td>
<td>50 - 100</td>
<td>none-poor</td>
<td>yellow</td>
<td>18 - 24 hrs</td>
</tr>
<tr>
<td><em>Salmonella Enteritidis</em> ATCC 13076</td>
<td>50 - 100</td>
<td>luxuriant</td>
<td>red with black centre</td>
<td>18 - 24 hrs</td>
</tr>
<tr>
<td><em>Salmonella Paratyphi B</em> ATCC 8759</td>
<td>50 - 100</td>
<td>luxuriant</td>
<td>red with black centre</td>
<td>18 - 24 hrs</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 25923</td>
<td>50 - 100</td>
<td>none-poor</td>
<td>yellow</td>
<td>18 - 24 hrs</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> ATCC 9027</td>
<td>&gt;=10³</td>
<td>inhibited</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> ATCC 29212</td>
<td>&gt;=10³</td>
<td>inhibited</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td><em>E. coli + S. Typhimurium</em> (mixed culture)</td>
<td>50 - 100</td>
<td>luxuriant</td>
<td>red with black centre</td>
<td>18 - 72 hrs</td>
</tr>
</tbody>
</table>

Key : (*) Corresponding WDCM numbers.

**Storage and Shelf Life**

Store between 15-25°C. 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,6).

**Reference**

3. Indian Pharmacopoeia, 2018, Govt. of India, Ministry of Health and Family Welfare, New Delhi
In vitro diagnostic medical device

CE Marking

Storage temperature

15°C-25°C

Do not use if package is damaged

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