Rappaport Vassiliadis Soyabean Meal Broth

Intended use
Recommended as selective enrichment medium for the isolation of *Salmonella* species. The composition and performance criteria of this medium are as per the specifications laid down in ISO 6579-1:2017.

Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzymatic digest of soya</td>
<td>4.500</td>
<td>Soya peptone#</td>
<td>4.500</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>7.200</td>
<td>Sodium chloride</td>
<td>7.200</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate (KH₂PO₄ + K₂HPO₄)</td>
<td>1.440</td>
<td>Potassium dihydrogen phosphate</td>
<td>1.260</td>
</tr>
<tr>
<td>Magnesium chloride, hexahydrate</td>
<td>28.600</td>
<td>Dipotassium hydrogen phosphate</td>
<td>0.180</td>
</tr>
<tr>
<td>Malachite green, oxalate</td>
<td>0.036</td>
<td>Magnesium chloride, hexahydrate</td>
<td>28.600</td>
</tr>
<tr>
<td>Final pH (after sterilization)</td>
<td>5.2±0.2</td>
<td>Malachite green, oxalate</td>
<td>0.036</td>
</tr>
</tbody>
</table>

# - Equivalent to enzymatic digest of soya
**Formula adjusted, standardized to suit performance parameters

Directions
Suspend 26.58 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified / distilled water. Heat gently if necessary to dissolve the medium completely. Dispense into tubes or flasks as desired and sterilize by autoclaving at 115°C for 15 minutes.

Principle And Interpretation

Rappaport Vassiliadis Soyabean Meal Broth (RVSM) is as per the specification laid down in ISO 6579 for the selective enrichment medium for isolation of *Salmonella* (3). Rappaport Vassiliadis Soyabean Meal Broth (RVSM) is modification of the Rappaport Vassiliadis Enrichment Broth, revised by van Schothorst (6-8). Van Schothorst modified the original formula by addition of dipotassium hydrogen phosphate to buffer the medium and addition of magnesium chloride to enhance the reliability of enrichment broth. Peterz (5) et al have also emphasized the importance of the concentration of magnesium chloride in the final medium.

The test specimen is added to Buffered Peptone Water (M1494I) and incubated at 34-36°C for 16-20 hours. This pre-enriched Buffered peptone water culture is inoculated into RVSM Broth and incubated at 41.5 ± 1°C for 24 ± 3 hours and further subcultured on XLD Agar (M031I) and Brilliant Agar w/ Phosphates (M016) or Bismuth sulphite Agar (M027). Further confirmation is carried out by isolation and biochemicals.

The medium contains soya peptone which provides essential growth nutrients. Magnesium chloride raises the osmotic pressure in the medium. Malachite green is inhibitory to organisms other than Salmonellae. The low pH of the medium, combined with the presence of malachite green and magnesium chloride, helps to select for the highly resistant *Salmonella* species. Phosphates buffer the medium to maintain the constant pH. Sodium chloride maintains the osmotic balance.

Type of specimen
Food, milk and milk products, animal feed, environmental samples

Specimen Collection and Handling
For food, milk and milk products, animal feed, environmental samples, follow appropriate techniques for sample collection and processing as per guidelines (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:
1. Due to nutritional variations some strains may show poor growth.

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
Blue coloured clear solution without any precipitate.

Reaction
Reaction of 2.66% w/v aqueous solution at 25°C. pH : 5.2±0.2

pH
5.00-5.40

Cultural Response
Cultural characteristics observed after an incubation at 41.5 ±1°C for 24 ± 3 hours. Further subculture is carried out on XLD Agar, Modified (M031I) and incubated at 37 ±1°C for 24 ± 3 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Recovery on XLD Agar (M031I)</th>
<th>Colour of colony on XLD Agar (M031I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella Enteritidis ATCC 13076 (00030*)+</td>
<td>50-100</td>
<td>&gt;10 colonies</td>
<td>red colonies w/ black centre</td>
</tr>
<tr>
<td>Esccherichia coli ATCC 8739 (00012*) +</td>
<td>&gt;10⁴</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa ATCC 27853 (00025*)</td>
<td>&gt;10⁴</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salmonella Typhimurium ATCC 14028 (00031*)+</td>
<td>50-100</td>
<td>&gt;10 colonies</td>
<td>red colonies w/ black centre</td>
</tr>
<tr>
<td>Esccherichia coli ATCC 25922 (00013*)+</td>
<td>&gt;10⁴</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa ATCC 27853 (00025*)</td>
<td>&gt;10⁴</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Selectivity
Cultural characteristics observed after an incubation at 41.5 ±1°C for 24 ± 3 hours. Further subculture is carried out on Tryptone Soya Agar (M290) and incubated at 37 ±1°C for 24 ± 3 hours.

| Esccherichia coli ATCC 8739 (00012*) | >10⁴ | partial inhibition | <=100 colonies on Tryptone Soya Agar |
| Esccherichia coli ATCC 25922 (00013*) | >10⁴ | partial inhibition | <=100 colonies on Tryptone Soya Agar |

Please refer disclaimer Overleaf.
**Storage and Shelf Life**

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

**Reference**


* - Corresponding WDCM Numbers

<table>
<thead>
<tr>
<th>Enterococcus faecalis</th>
<th>ATCC 29212(00087*)</th>
<th>&gt;10⁴ colonies on Tryptone Soya Agar</th>
<th>&lt;10 colonies on Tryptone Soya Agar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococcus faecalis</td>
<td>ATCC 19433 (000094*)</td>
<td>&gt;10⁴ colonies on Tryptone Soya Agar</td>
<td>&lt;10 colonies on Tryptone Soya Agar</td>
</tr>
</tbody>
</table>

**Disclaimer**

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