Semisolid Rappaport Vassiliadis Medium, Modified

Intended Use
Recommended for the detection of motile *Salmonella* species from food, faeces and environmental specimens.

Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptose</td>
<td>4.590</td>
</tr>
<tr>
<td>Tryptone</td>
<td>4.590</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>7.340</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>1.470</td>
</tr>
<tr>
<td>Magnesium chloride, anhydrous</td>
<td>10.930</td>
</tr>
<tr>
<td>Malachite green oxalate</td>
<td>0.037</td>
</tr>
<tr>
<td>Agar</td>
<td>2.700</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>5.2±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters

Directions
Suspend 31.66 grams in 1000 ml purified/distilled water. Heat to boiling with frequent agitation to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C and aseptically add the rehydrated contents of 1 vial of Novobiocin Selective Supplement (FD290) or 4 vials of Novobiocin supplement (FD096). Mix well and pour into sterile Petri plates. Air dry the plated medium at room temperature for at least one hour.

Principle And Interpretation
Semisolid Rappaport Vassiliadis Medium, Modified is prepared as per the formulation described by DeSmedt et al (2), for the detection of motile *Salmonella* species from food and environmental specimens. This medium detects more *Salmonella* positive samples than the routinely used enrichment procedures (3,4,5).

Tryptose, tryptone provide the nitrogenous and carbonaceous substances and other essential growth nutrients. Phosphate gives good buffering capacity to the medium. Malachite green oxalate and Novobiocin inhibits many gram positive bacteria. The working of medium is based on the ability of *Salmonella* species to migrate in the selective medium by competing with the other motile organisms, thus producing opaque halos of growth. This medium can be used in combination with direct culture and Selenite F Broth (M052A) enrichment for isolation of *Salmonella* species from faeces and sub-culturing on XLD Agar (M031) or Mannitol Lysine Agar (M1071) results in higher recovery rates (1). This medium is not suitable for the detection of non-motile strains of *Salmonella* (6).

Inoculate 3 drops (0.1 ml) of pre-enrichment culture (16-20 hours old) in separate spots on the air-dried medium surface. Incubate the plates in an upright position at 42°C for up to 24 hours. The motile bacteria will show a halo or zone of growth originating from inoculation spot. *Salmonella* species show straw coloured colonies. Sub-cultures can be carried out from the outside edge of the halo to confirm purity and for further biochemical and serological tests.

Type of specimen
Clinical samples - faeces, Food samples: meat and meat products.

Specimen Collection and Handling:
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7,8).
For food samples, follow appropriate techniques for sample collection and processing as per guidelines (9).
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 variable nutritional requirements, some strains show poor growth on this medium.
2. The plates should be incubated in upright and steady position as medium contains less agar.
3. Air dried plate should be used, or it may give erroneous results.

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

Gelling
Semisolid, comparable with 0.27% Agar gel.

Colour and Clarity of prepared medium
Greenish blue coloured clear to slightly opalescent semisolid gel forms in Petri plates which may have a slight precipitate.

Reaction
Reaction of 3.16% w/v aqueous solution at 25°C. pH : 5.2 ± 0.2

pH
5.00-5.40

Cultural Response
M1282: Cultural characteristics observed after an incubation at 42-43°C for 18-24 hours with added Novobiocin Selective Supplement (FD290) or Novobiocin supplement (FD096).

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth at 42±1°C</th>
<th>Motility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrobacter freundii ATCC</td>
<td>8090 (00006*)</td>
<td>50-100</td>
<td>none-poor, negative reaction, no colour change</td>
</tr>
<tr>
<td>Escherichia coli ATCC</td>
<td>25922 (00013)*</td>
<td>50-100</td>
<td>none-poor, negative reaction, no colour change</td>
</tr>
<tr>
<td>Salmonella Typhi ATCC</td>
<td>6539</td>
<td>50-100</td>
<td>good-luxuriant positive reaction, colourless to light pink zone</td>
</tr>
<tr>
<td>Salmonella Typhimurium ATCC</td>
<td>14028 (00031*)</td>
<td>50-100</td>
<td>good-luxuriant positive reaction, colourless to light pink zone</td>
</tr>
<tr>
<td>Salmonella Enteritidis ATCC</td>
<td>13076 (00030*)</td>
<td>50-100</td>
<td>good-luxuriant positive reaction, colourless to light pink zone</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 inorder 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 (7, 8).

Reference


Disclaimer:

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