Rappaport-Vassiliadis R10 Broth

**Intended Use**
Rappaport-Vassiliadis R10 Broth is used for selectively enriching *Salmonella* from meat and dairy products, feces and sewage-polluted water.

**Summary and Explanation**
Rappaport et al. formulated an enrichment medium for *Salmonella* that was modified by Vassiliadis et al. The Rappaport formulation, designated R25/37°C, recommended incubation at 37°C; the Vassiliadis modification, designated R10/43°C, had a reduced level of malachite green and recommended incubation at 43°C. Later work by Peterz showed that incubation at 41.5°C ± 0.5°C for 24 hours improved recovery of *Salmonella* spp.

Rappaport-Vassiliadis R10 Broth is a selective enrichment medium that is used following pre-enrichment of the specimen in a suitable pre-enrichment medium. It has gained approval for use in analyzing milk and milk products, raw flesh foods, highly contaminated foods and animal feeds.

**Principles of the Procedure**
Rappaport-Vassiliadis R10 Broth contains peptone as the carbon and nitrogen source for general growth requirements. 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* spp.

**Formula**

**Difco™ Rappaport-Vassiliadis R10 Broth**

Approximate Formula* Per Liter

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic Digest of Casein</td>
<td>4.54 g</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>7.2 g</td>
</tr>
<tr>
<td>Monopotassium Phosphate</td>
<td>1.45 g</td>
</tr>
<tr>
<td>Magnesium Chloride (anhydrous)</td>
<td>13.4 g</td>
</tr>
<tr>
<td>Malachite Green Oxalate</td>
<td>36.0 mg</td>
</tr>
</tbody>
</table>

*Adjusted and/or supplemented as required to meet performance criteria.

**Directions for Preparation from Dehydrated Product**
1. Suspend 26.6 g of the powder in 1 L of purified water. Mix thoroughly.
2. Warm slightly to completely dissolve the powder.
3. Dispense 10 mL amounts into suitable containers.
4. Autoclave at 116°C (10 psi pressure) for 15 minutes.
5. Test samples of the finished product for performance using stable, typical control cultures.

**Procedure and Expected Results**

**Water and Sewage Samples**
For isolating *Salmonella* (other than *S. Typhi*) from water and associated materials, such as sewage liquor, sewage sludge, digested sludge and pressed sludge cake:

1. Concentrate the sample by filtering it through a plug of sterile absorbent cottonwool inserted in the neck of a large sterile funnel or through a Whatman No. 17 absorbent pad.
Pre-enrichment

2. Using aseptic technique, transfer the cottonwool plug or the pad to 100 mL of a suitable pre-enrichment medium such as Buffered Peptone Water.
3. Incubate at 37 ± 0.5°C for 18-24 hours.

Selective Enrichment

4. Inoculate 10 mL of Rappaport-Vassiliadis R10 Broth with 0.1 mL of the pre-enrichment culture. Inoculate 10 mL of Muller-Kauffman Tetrathionate Broth with 1 mL of the pre-enrichment culture.
5. Incubate Rappaport-Vassiliadis R10 Broth at 41.5 ± 0.5°C. Incubate Muller-Kauffman Tetrathionate Broth at 42 ± 1°C for 48 hours.

Expected Results

6. After incubation, subculture both selective enrichment broths to Brilliant Green Agar and XLD Agar. Incubate at 35 ± 2°C for 18-24 hours.

Milk and Foods

For isolating Salmonella (other than S. Typhi) from milk and milk products,4 raw flesh foods, highly contaminated foods and animal feeds:5

Pre-enrichment

1. Add 25 g or a 25 mL sample of the specimen to 225 mL of pre-enrichment medium. Consult appropriate references for the type of product being tested.5,6
2. Incubate at 35 ± 2°C for 20-24 hours5 or at 37°C for 16-20 hours5 depending on the referenced procedure being followed.

Selective Enrichment

3. Inoculate 10 mL of Rappaport-Vassiliadis R10 Broth with 0.1 mL of pre-enrichment culture. Inoculate 10 mL of another selective enrichment medium such as Tetrathionate Broth or Selenite Cystine Broth with the recommended amount of pre-enrichment culture.4,5
4. Incubate Rappaport-Vassiliadis R10 Broth at 41.5 ± 0.5°C for 24 ± 2 hours or at 42 ± 0.5°C for 22-24 hours.5 Incubate the other selective enrichment broths appropriately.

Expected Results

5. After incubation, subculture Rappaport-Vassiliadis R10 Broth and the other selective enrichment broths to selective agar media and incubate at 35 ± 2°C for 24 ± 2 hours4 or for 18-24 hours.5
6. Examine for typical Salmonella colonies. Confirm identification of isolates by biochemical and serologic tests.4,5

Limitation of the Procedure

The combined inhibitory factors of this medium (malachite green, magnesium chloride, low pH) may inhibit certain Salmonella, such as Salmonella Typhi and S. Paratyphi A. Isolation techniques should include a variety of enrichment broths and isolation media.

References


Availability

Difco™ Rappaport-Vassiliadis R10 Broth

<table>
<thead>
<tr>
<th>IDF</th>
<th>ISO</th>
<th>USDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat. No.</td>
<td>218581</td>
<td>Dehydrated – 500 g</td>
</tr>
<tr>
<td>Europe</td>
<td>Cat. No.</td>
<td>257257</td>
</tr>
</tbody>
</table>

Difco™ & BBL™ Manual, 2nd Edition