INTENDED USE

BD Rappaport Vassiliadis Broth (=R10 Broth) is a liquid medium for selectively enriching Salmonella from meat and dairy products, feces and polluted water.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

Rappaport et al. formulated an enrichment medium for Salmonella that was modified by Vassiliadis et al. 1,2 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° ± 0.5°C for 24 hours improved recovery of Salmonella spp. 3 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, 4 raw meat foods, highly contaminated foods and animal feeds. 5,6 The medium is also recommended as a selective enrichment of Salmonella other than Salmonella Typhi from human stool specimens. 7,8

In BD Rappaport Vassiliadis Broth, Tryptone is a source of carbon and nitrogen 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 (5.1±0.2), combined with the presence of malachite green and the high concentration of magnesium chloride which increases the osmotic pressure, select for Salmonella spp.

REAGENTS

BD Rappaport Vassiliadis Broth

Formula* Per Liter Purified Water

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto™ Tryptone</td>
<td>4.54 g</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>7.2</td>
</tr>
<tr>
<td>Potassium Dihydrogen Phosphate</td>
<td>1.45</td>
</tr>
<tr>
<td>Magnesium Chloride, anhydrous</td>
<td>13.4</td>
</tr>
<tr>
<td>Malachite Green Oxalate</td>
<td>0.036</td>
</tr>
</tbody>
</table>

pH 5.1 ±0.2

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

PRECAUTIONS

For professional use only.

Do not use vials if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

Consult GENERAL INSTRUCTIONS FOR USE document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE

On receipt, store vials in the dark at 2 to 8°C until just prior to use. Avoid freezing and overheating. The vials may be inoculated up to the expiration date (see container or package label) and incubated for the recommended incubation times.

Vials from opened packages can be used up to the expiration date. Opened vials must be used immediately.
**USER QUALITY CONTROL**

Inoculate representative samples with the following strains (for details, see GENERAL INSTRUCTIONS FOR USE document). Incubate 18 to 48 hours at 41,5°C ± 0,5°C. Subculture onto appropriate solid selective media, e.g. BD Brilliant Green Agar. Incubate the plates for 18 to 48 hours at 35 – 37°C.

<table>
<thead>
<tr>
<th>Test strain</th>
<th>Turbidity</th>
<th>Growth results on BD Brilliant Green Agar (subculture)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella Typhimurium</em> ATCC™ 14028</td>
<td>Strong</td>
<td>Good to excellent</td>
</tr>
<tr>
<td><em>Salmonella Enteritidis</em> ATCC 13076</td>
<td>Strong</td>
<td>Good to excellent</td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 25922</td>
<td>None to light</td>
<td>Inhibition partial (to complete)</td>
</tr>
<tr>
<td>Uninoculated</td>
<td>Blue, clear</td>
<td></td>
</tr>
</tbody>
</table>

**PROCEDURE**

**Materials Provided**

BD Rappaport Vassiliadis Broth, provided in 30 ml screw-cap vials. Microbiologically controlled.

**Materials Not Provided**

Ancillary culture media, reagents and laboratory equipment as required.

**Specimen Types**

This medium is used for the isolation of *Salmonella* other than *Salmonella Typhi* from foods and from fecal specimens (see also PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE).

**Test Procedure**

**Feces:** Inoculate 10 ml of Rappaport-Vassiliadis Broth with a heavy loopful of feces or 50 to 100 µl of liquid stools.

**Foods:** Inoculate 10 ml of Rappaport-Vassiliadis Broth with 0.1 ml of the pre-enrichment culture (e.g. Buffered Peptone Water).

Milk and milk products, raw meat, highly contaminated foods and animal feeds: Follow the appropriate guidelines.

Strongly contaminated materials such as sewage or sewage sludge must be filtered through cotton wool to remove undissolved material before inoculating Rappaport-Vassiliadis Broth.

Incubate Rappaport-Vassiliadis Broth at 41.5 +/- 0.5°C for 18 to 48 hours.

**Results**

After incubation, growth may be detected by a milky appearance of the medium or by turbidity.

Since a clear medium is not always negative for bacterial growth, a subculture should always be performed onto solid media, e.g., BD Brilliant Green Agar, BD XLD Agar, or on other suitable *Salmonella* media. Use of less selective media, e.g., BD MacConkey II Agar, is also recommended. Incubate at 35 ± 2°C for 18-24 hours or longer if required. Suspicious colonies obtained on the solid media must be further identified by serological and biochemical methods.

**PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**

BD Rappaport Vassiliadis Broth is used as a selective enrichment medium for *Salmonella* from human feces and a variety of nonclinical materials such as foods and sewage.

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

Media for subculture from BD Rappaport Vassiliadis Broth should include a less selective one, e.g., BD MacConkey II Agar.

Biochemical and serological identification of the isolates obtained after subculture is necessary.
REFERENCES

PACKAGING/AVAILABILITY
Cat. No. 257257  Ready-to-use Bottled Medium  cpu 50; 10 ml in 30 ml screw cap vials

FURTHER INFORMATION
For further information please contact your local BD representative.

BD Diagnostic Systems
Tullastrasse 8 – 12
D-69126 Heidelberg/Germany
Phone: +49-62 21-30 50  Fax: +49-62 21-30 52 16
Reception_Germany@europe.bd.com

BD Diagnostic Systems Europe
Becton Dickinson France SA
11 rue Aristide Bergès
38800 Le Pont de Claix/ France
Tel: +33-476 68 3636  Fax: +33-476 68 3292  http://www.bd.com

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