



BD™ Rappaport Vassiliadis Broth

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 $41.5^\circ \pm 0.5^\circ\text{C}$ 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

Bacto™ Tryptone	4.54 g
Sodium Chloride	7.2
Potassium Dihydrogen Phosphate	1.45
Magnesium Chloride, anhydrous	13.4
Malachite Green Oxalate	0.036

pH 5.1 +/- 0.2

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

PRECAUTIONS

IVD . 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.

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

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 guidelines⁴⁻⁶.

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.⁴⁻⁸

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

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2. Vassiliadis, P., D. Trichopoulos, A. Kalandidi, and E. Xirouchaki. 1978. Isolation of salmonellae from sewage with a new procedure of enrichment. *J. Appl. Bacteriol.* 44:233-239.
3. Peterz, M., C. Wiberg, and P. Norberg. 1989. The effect of incubation temperature and magnesium chloride concentration on growth of salmonella in home-made and commercially available dehydrated Rappaport-Vassiliadis broths. *J. Appl. Bacteriol.* 66:523-528.
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7. Kist, M., et al. 2000. Infektionen des Darmes. *In: Mauch, H., Lüttiken, R., and S. Gatermann (eds.): MiQ - Qualitätsstandards in der mikrobiologisch-infektiologischen Diagnostik, vol. 9.* Urban & Fischer, Munich, Germany.
8. Bockemühl, J. 1992. *Enterobacteriaceae*. *In: Burkhardt, F. (ed.). Mikrobiologische Diagnostik.* Thieme Verlag, Stuttgart, New York.

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.



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