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.[1] formulated an enrichment medium for *Salmonella* that was modified by Vassiliadis et al.[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 flesh foods, highly contaminated foods and animal feeds.[5]

User Quality Control

Identity Specifications

Difco™ Rappaport-Vassiliadis R10 Broth

Dehydrated Appearance:	Pale green to green, free-flowing, homogeneous.
Solution:	2.66% solution, soluble in purified water upon gentle heating. Solution is blue, clear.
Prepared Appearance:	Blue, clear.
Reaction of 2.66% Solution at 25°C:	pH 5.1 ± 0.2

Cultural Response

Difco™ Rappaport-Vassiliadis R10 Broth

Prepare the medium per label directions. Inoculate and incubate at 41.5 ± 0.5°C for 18-48 hours. Subculture to Brilliant Green Agar and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	10^3-2×10^3	Marked inhibition
Proteus mirabilis	9240	10^3-2×10^3	Marked inhibition
Salmonella enterica subsp. *enterica* serotype Enteritidis	13076	10^2-10^3	Good
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^2-10^3	Good

This medium selectively enriches for salmonellae because bacteria, including other intestinal bacteria, are typically inhibited by malachite green, high osmotic pressure and/or low pH. *Salmonella* Typhi and *S.* Paratyphi A are sensitive to malachite green and may be inhibited.

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		
Pancreatic Digest of Casein	4.54	g
Sodium Chloride	7.2	g
Monopotassium Phosphate	1.45	g
Magnesium Chloride (anhydrous)	13.4	g
Malachite Green Oxalate	36.0	mg

**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.
7. Examine for typical *Salmonella* colonies. Confirm identification of isolates by biochemical and serologic tests.

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.[4,5]
2. Incubate at 35 ± 2°C for 20-24 hours[5] or at 37°C for 16-20 hours,[4] 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[4] 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 hours[4] 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

1. Rappaport, Konforti and Navon. 1956. J. Clin. Pathol. 9:261.
2. Vassiliadis, Trichopoulos, Kalandidi and Xirouchaki. 1978. J. Appl. Bacteriol. *44*:233.
3. Peterz, Wiberg and Norberg. 1989. J. Appl. Bacteriol. *66*:523.
4. International Organization for Standardization. 2001. Milk and milk products – detection of *Salmonella*. ISO 6785/IDF 93:2001. ISO, Geneva, Switzerland.
5. U.S. Department of Agriculture. Microbiology laboratory guidebook, online. Food Safety and Inspection Service, USDA, Washington, D.C.

Availability

Difco™ Rappaport-Vassiliadis R10 Broth

IDF ISO USDA

Cat. No. 218581 Dehydrated – 500 g

Europe

Cat. No. 257257 Prepared Tubes, 10 mL – Ctn. of 50