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]

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.

PRINCIPLE

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.

REAGENTS (FORMULA)

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
Deionized Water	1000.0	ml

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 hours5 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]

QUALITY CONTROL

All lot numbers have been tested and have been found to be acceptable. Customers can test products using the following quality control organisms. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, sample results should not be reported.

Organisms	Incubation	Results
Salmonella enterica subsp. *Enterica* serotype Typhimurium ATCC 14028	41.5 ± 5°C for 18-48 hours	Growth
Escherichia coli ATCC 25922	41.5 ± 5°C for 18-48 hours	Inhibition

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BIBLIOGRAPHY

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.

Viikinkaari 6, 00790 Helsinki Room 306A5

Phone: +358 (45) 8016507 Email: info@abbadis-life.com Website: https://abbadis-life.com