



RAPPAPORT-VASSILIADIS R10 BROTH (7512)

Intended Use

Rappaport-Vassiliadis R10 Broth is used for the selective enrichment of *Salmonella* spp. from foods.

Product Summary and Explanation

Rappaport et al¹ formulated an enrichment medium for *Salmonella* spp. that was modified by Vassiliadis et al.² The Rappaport formulation, designated R 25/37°C, recommended incubation at 37°C. The Vassiliadis modification, designated R 10/43°C, had a reduced level of Malachite Green and recommended incubation at 43°C. Peterz later 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. It has gained approval for use in analyzing milk and milk products,⁴ raw flesh foods, highly contaminated foods, and animal feeds.^{5,6} This medium selectively enriches for *Salmonella* spp. because bacteria, including other intestinal bacteria, are typically susceptible to or inhibited by Malachite Green, high osmotic pressure and/or low pH. *S. typhi* and *S. choleraesuis* are sensitive to Malachite Green and may be inhibited.

Principles of the Procedure

Enzymatic Digest of Casein is the carbon and nitrogen sources for general growth requirements in Rappaport-Vassiliadis R10 Broth. Magnesium Chloride raises the osmotic pressure in the medium, and Potassium Dihydrogen Phosphate acts as a buffer. Malachite Green Oxalate is inhibitory to organisms other than *Salmonella* spp. The low pH of the medium, combined with the presence of Malachite Green Oxalate and Magnesium Chloride, select for the highly resistant *Salmonella* spp.

Formula / Liter

Enzymatic Digest of Casein.....	4.54 g
Sodium Chloride.....	7.20 g
Potassium Dihydrogen Phosphate.....	1.45 g
Magnesium Chloride, Anhydrous.....	13.4 g
Malachite Green Oxalate.....	0.036 g

Final pH: 5.1 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Dissolve 26.6 g of the medium in one liter of purified water.
2. Mix thoroughly.
3. Autoclave at 116°C (10 lb pressure) for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and pale green.

Prepared Appearance: Prepared medium is clear, may have a slight precipitate and dark turquoise.

Expected Cultural Response: Cultural response in Rappaport-Vassiliadis R10 Broth incubated aerobically inoculated at 41.5 ± 0.5°C for 18 - 24 hours. After incubation, subculture to Brilliant Green Agar and incubated at 35 ± 2°C and examined for growth on Brilliant Green Agar for 18 - 24 hours.

Microorganism	Approx. Inoculum (CFU)	Response
<i>Escherichia coli</i> ATCC® 25922	~ 1000	Inhibited
<i>Proteus vulgaris</i> ATCC® 13315	~ 1000	Inhibited
<i>Salmonella arizonae</i> ATCC® 13314	10 - 100	Growth, pink colonies
<i>Salmonella typhimurium</i> ATCC® 14028	10 - 100	Growth, pink colonies

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

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

Selective Enrichment: Inoculate 10 mL of R-V 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. Incubate Rappaport-Vassiliadis R10 Broth at 41.5 ± 0.5°C. Incubate Muller Kauffman Tetrathionate Broth at 42 ± 1°C for 48 hours.

Results: After incubation, subculture both selective enrichment broths to Brilliant Green Agar and XLD Agar. Incubate at 35°C for 18 - 24 hours. Examine for typical *Salmonella* colonies. Confirm identification of isolates through biochemical and serological testing.

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.^{5,6}

Pre-Enrichment: 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.^{4,5,6} Incubate at 35°C for 24 ± 2 hours^{5,6} or at 37°C for 16 - 20 hours,⁴ depending on the referenced procedure being followed.

Selective Enrichment: 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 1 mL of the pre-enrichment culture.^{4,5,6} Incubate Rappaport-Vassiliadis R10 Broth at 41.5 ± 0.5°C for 24 ± 2 hours. Incubate the other selective enrichment broths appropriately.

Results: 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.^{4,5} Examine for typical *Salmonella* colonies. Confirm identification of isolates through biochemical and serologic tests.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container.

Limitation of the Procedure

The combined inhibitory factors of this medium may inhibit certain *Salmonella*, such as *S. typhi* and *S. choleraesuis*. Isolation techniques should include a variety of enrichment broths and isolation media.

Packaging

Rappaport-Vassiliadis R10 Broth	Code No.	7512A	500 g
		7512B	2 kg
		7512C	10 kg

References

1. Rappaport, F., N. Konforti, and B. Navon. 1956. A new enrichment medium for certain salmonellae. J. Clin. Pathol. **9**:261-266.
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 homemade and commercially available dehydrated Rappaport-Vassiliadis broths. J. Appl. Bacteriol. **66**:523-528.
4. International Dairy Federation. 1995. Milk and milk products: detection of *Salmonella*. IDF Standard **93B**:1005. Brussels, Belgium.
5. Cunniff, P. (ed.). 1995. Official Methods of Analysis AOAC International, 16th ed., AOAC International, Gaithersburg, MD.
6. www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.