

Rappaport Vassiliadis R10 Broth (NCM0114)

Intended Use

Rappaport Vassiliadis R10 Broth is used for the selective enrichment of *Salmonella* spp. from foods in a laboratory setting. Rappaport Vassiliadis R10 Broth is not intended for use in the diagnosis of disease or other conditions in humans.

Description

Rappaport et al formulated an enrichment medium for *Salmonella* spp. that was modified by Vassiliadis et al. 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 $41.5 \pm 0.5^\circ\text{C}$ for 24 hours improved recovery of *Salmonella* spp. 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, raw flesh foods, highly contaminated foods, and animal feeds. 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.

Typical Formulation

Enzymatic Digest of Casein	4.5 g/L
Sodium Chloride	7.2 g/L
Potassium Dihydrogen Phosphate	1.4 g/L
Magnesium Chloride, Anhydrous	13.4 g/L
Malachite Green Oxalate	0.036 g/L

Final pH: 5.1 ± 0.2 at 25°C

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

Precaution

Refer to SDS

Preparation

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 $41.5 \pm 0.5^\circ\text{C}$ for 18 - 24 hours. After incubation, subculture to Brilliant Green Agar and incubated at $35 \pm 2^\circ\text{C}$ and examined for growth on Brilliant Green Agar for 18 - 24 hours.

Microorganism	Approx. Inoculum (CFU)	Response
<i>Escherichia coli</i> ATCC® 25922	~ 1000	Completely Inhibited
<i>Proteus vulgaris</i> ATCC® 13315	~ 1000	Completely 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 $41.5 \pm 0.5^\circ\text{C}$. Incubate Muller Kauffman Tetrathionate Broth at $42 \pm 1^\circ\text{C}$ 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.

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. Incubate at 35°C for 24 ± 2 hours or at 37°C for 16 - 20 hours, 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. Incubate Rappaport-Vassiliadis R10 Broth at $41.5 \pm 0.5^\circ\text{C}$ 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 $35 \pm 2^\circ\text{C}$ for 24 ± 2 hours.⁴ Examine for typical *Salmonella* colonies. Confirm identification of isolates through biochemical and serologic tests.

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.

Storage

Store dehydrated culture media at 2-30°C away from direct sunlight. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

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.

Technical Specification Sheet



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. Cunnif, P. (ed.). 2016. Official Methods of Analysis AOAC International, 20thed., AOAC International, Gaithersburg, MD.
6. www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.html.

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