RAPPAPORT-VASSILIADIS SALMONELLA ENRICHMENT BROTH (7730)

Intended Use

Rappaport-Vassiliadis Salmonella Enrichment Broth is used for the enrichment and selective isolation of *Salmonella* spp. Conforms to Harmonized USP/EP/JP Requirements[1,2,3] in a laboratory setting. Rappaport-Vassiliadis Salmonella Enrichment Broth is not intended for use in the diagnosis of disease or other conditions in humans.

Product Summary and Explanation

Rappaport et al[4] formulated an enrichment medium for *Salmonella spp.* that was modified by Vassiliadis et al.[5] 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.[6]

Rappaport-Vassiliadis Salmonella Enrichment Broth is a modification of Rappaport-Vassiliadis R10 Broth, where Soy Peptone has replaced Enzymatic Digest of Casein as the nitrogen and vitamin source. Soy Peptone has shown to enhance the growth of *Salmonella* spp.[7,8] and minimizes Bovine Spongiform Encephalopathy (BSE) risk. Rappaport-Vassiliadis Salmonella Enrichment Broth conforms to Harmonized United States Pharmacopoeia (USP), European Pharmacopoeia (EU), and Japanese Pharmacopoeia (JP).[1,2,3] 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

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

Formula / Liter

Soy Peptone	4.50 g
Sodium Chloride	8.0 g
Potassium Phosphate, monobasic	0.60 g
Potassium Phosphate, dibasic	0.40 g
Magnesium Chloride, anhydrous*	13.58 g
Malachite Green	0.036 g

Final pH: 5.2 ± 0.2 at 25°C

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

* Equivalent to 29.0 g/L Magnesium Chloride hexahydrate

Precautions

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

Directions

1. Dissolve 27.2 g of the medium in one liter of purified water.
2. Mix thoroughly.
3. Dispense 10 mL into glass tubes, cap and autoclave at 115°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige to light green.

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

Expected Cultural Response and USP/EP/JP Growth Promotion Testing: Cultural response with organisms grown in Buffered Peptone Water at 35 ± 2°C for 20 hours. The cultures were incubated aerobically in Rappaport-Vassiliadis Salmonella Enrichment Broth at 41.5 ± 0.5°C for 21 – 27 hours. After incubation at Harmonized USP/EP/JP specified temperatures and incubation times, organisms were subcultured to XLD ISO Agar (9207) and incubation at 35 ± 2°C, the plates were examined for growth at 18 – 24 hours. [1,2,3]

Microorganism	Approx. Inoculum (CFU)	Expected Results Recovery on XLD Agar
Salmonella typhimurium ATCC® 14028	BPW, 20 hours	Growth, red colonies, black centers
Salmonella enteritidis ATCC® 13076	BPW, 20 hours	Growth, red colonies, black centers
Salmonella arizonae ATCC® 13314	BPW, 20 hours	Growth, red colonies, black centers
Escherichia coli ATCC® 8739	BPW, 20 hours	Inhibited to suppressed, yellow colonies
Escherichia coli ATCC® 25922	BPW, 20 hours	Inhibited to suppressed, yellow colonies
Pseudomonas aeruginosa ATCC® 27853	BPW, 20 hours	Suppressed, red colonies
Enterococcus faecalis ATCC® 19433	BPW, 20 hours	Inhibited
Enterococcus faecalis ATCC® 29212	BPW, 20 hours	Inhibited
Staphylococcus aureus ATCC® 25923	BPW, 20 hours	Inhibited

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

Test Procedure

Food and Environmental Specimens

1. Prepare Buffered Peptone Water (7418) or Buffered Peptone Water (ISO), pH 7.0 (9176) pre label directions and dispense 225 mL of the medium into appropriate container.
2. Prepare Rappaport-Vassiliadis Salmonella Enrichment Broth following product label.
3. Add 25 g of the test specimen to 225 mL of Buffered Peptone Water and incubate at 35°C for 16 – 20 hours.
4. Inoculate 0.1 mL of the pre-enrichment Buffered Peptone Water to 10 mL of Rappaport-Vassiliadis Salmonella Enrichment Broth and incubate at 41.5 ± 0.5°C for 24 – 48 hours.
5. Subculture the broth by streaking on to prepared XLD Agar (ISO). Incubate at 35 ± 2°C, the plates were examined for growth at 18 – 24 hours.

Results

Suspect colonies showing typical *Salmonella* morphology, good growth of red colonies with black centers, should be confirmed by biochemical and/or serological procedures.

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

1. 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.
2. Incubation temperature of this procedure is critical. To allow for incubator temperature fluctuation, 42 ± 0.1°C is preferred recommendation.

Packaging

Rappaport-Vassiliadis Salmonella Enrichment Broth	**Code No.**	**7730A 500 g**
		7730B 2 kg
		7730C 10 kg

References

1. **United States Pharmacopeial Convention.** 2007. The United States pharmacopeia, Amended Chapters 61, 62, 111. The United States Pharmacopeial Convention, Rockville, MD.
2. **Directorate for the Quality of Medicines of the Council of Europe (EDQM).** 2007. The European Pharmacopoeia, Amended Chapters 2.6.12, 2.6.13, 5.1.4, Council of Europe, 67075 Strasbourg Cedex, France.
3. **Japanese Pharmacopoeia.** 2007. Society of Japanese Pharmacopoeia. Amended Chapters 35.1, 35.2, 7. The Minister of Health, Labor, and Welfare.
4. **Rappaport, F., N. Konforti, and B. Navon.** 1956. A new enrichment medium for certain salmonellae. J. Clin. Pathol. **9:**261-266.
5. **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.
6. **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.
7. **vanSchothorst, M. and A. M. Renaud.** 1983. J. Appl. Bact. **54:**209-215.
8. **McGibbon, L., E. Quail, and C. R. Fricker.** 1984. Inter. J. Food Microbiol. **1:**171-177.

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.

NEOGEN CORPORATION
620 Lesher Place, Lansing MI 48912
517/372-9200 • 800/783-3212 • fax: 800/875-8563
neogen-info@neogen.com • www.neogen.com