

Rappaport Vassiliadis Salmonella Enrichment Broth (NCM0103)

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. Rappaport-Vassiliadis Salmonella Enrichment Broth is not intended for use in the diagnosis of disease or other conditions in humans.

Description

A medium recommended by the Harmonized Pharmacopoeia for the enrichment and selective isolation of *Salmonella* spp. from non-sterile samples. Malachite green and magnesium chloride act as selective agents, combined with high osmotic pressure and low pH to effectively inhibit non-target competitive organisms. Soya peptone provides a source of nitrogen and potassium phosphate acts as a buffer. Some *Salmonella* (*S. typhi* and *S. enteritidis*) are known to be sensitive to malachite green and as such may fail to grow. If these organisms are suspected an alternative selective enrichment broth (e.g. NCM0126 Mueller-Kauffmann Tetrathionate novobiocin Broth - MKTTn) should be used in parallel. This formulation is hygroscopic and will produce a slight exothermic reaction when mixed with water. According to the Harmonized Pharmacopoeia, Rappaport Vassiliadis Salmonella Enrichment Broth is used as a selective enrichment broth, with subculture performed onto Xylose Lysine Deoxycholate (XLD) agar (NCM0027).

Typical Formulation

Soy Peptone	4.5 g/L
Sodium Chloride	8.0 g/L
Potassium Phosphate, Monobasic	0.6 g/L
Dipotassium Phosphate	0.4 g/L
Magnesium Chloride, Anhydrous*	13.58 g/L
Malachite Green	0.036 g/L

Final pH: 5.2 ± 0.2 at 25°C

* Equivalent to 29.0 g/L Magnesium Chloride hexahydrate

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

Precaution

Refer to SDS

Preparation

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.

Test Procedure

Food and Environmental Specimens

1. Prepare Buffered Peptone Water (NCM0003) or Buffered Peptone Water (ISO), (NCM0015) per label directions and dispense 225 mL of the medium into appropriate container.
2. Prepare Rappaport-Vassiliadis Salmonella Enrichment Broth following product label.
3. Add the appropriate sample volume per the required test method the appropriate volume of Tryptic Soy Broth (Soybean-Casein Digest Broth), (NCM0004) and incubate at 35°C for 16 – 20 hours.
4. Subculture the broth by streaking on to prepared XLD Agar (NCM0027). Incubate at 35 ± 2°C, the plates were examined for growth at 18 – 24 hours.

Technical Specification Sheet



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 aerobically in Rappaport-Vassiliadis Salmonella Enrichment Broth at 30-35°C for 18 – 24 hours. After incubation, the organisms were subcultured to XLD Agar (NCM0027) and incubation at 35 ± 2°C, the plates are examined for growth at 18 – 24 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results
		Recovery on XLD Agar
<i>Salmonella typhimurium</i> ATCC® 14028	10-100	Growth, red colonies, black centers
<i>Salmonella enteritidis</i> ATCC® 13076	10-100	Growth, red colonies, black centers
<i>Salmonella arizonae</i> ATCC® 13314	10-100	Growth, red colonies, black centers
<i>Salmonella abony</i> NCTC 6017	10-100	Growth, red colonies, black centers
<i>Escherichia coli</i> ATCC® 8739	~1000	Inhibited to suppressed, yellow colonies
<i>Pseudomonas aeruginosa</i> ATCC® 27853	~1000	Inhibited to suppressed, red colonies
<i>Enterococcus faecalis</i> ATCC® 29212	~1000	Inhibited
<i>Staphylococcus aureus</i> ATCC® 6538	~1000	Inhibited

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

Results

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

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. enteritidis*. 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.

Storage

Dehydrated Culture Media: 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.

References

1. European Pharmacopoeia 9th Edition (2017)
2. United States Pharmacopoeia National Formulary 2018: USP 41 NF 35
3. Japanese Pharmacopoeia 17th Edition (2017)
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.



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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. van Schothorst, 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.

Effective Date: 11/29/2018

Revision: 0



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