

RAPPAPORT-VASSILIADIS (MSRV) MEDIUM SEMISOLID MODIFIED (7511)

Intended Use

Rappaport-Vassiliadis (MSRV) Medium Semisolid Modified is used with Novobiocin for the rapid detection of motile *Salmonella* spp.

Product Summary and Explanation

Rappaport-Vassiliadis (MSRV) Medium Semisolid Modified is a modification of Rappaport-Vassiliadis enrichment broth for detecting motile *Salmonella* spp. in feces and food products.^{1,2} The original research on MSRV Medium revealed a semisolid could be used as a rapid and sensitive test for isolating motile *Salmonella* from food products following pre-enrichment or selective enrichment.^{3,4} The semisolid medium allows motility to be detected as halos of growth around the original point of inoculation.

MSRV Medium is recommended by the European Chocolate Manufacturer's Association. A collaborative study performed with support of American Cocoa Research Institute and the Canadian Chocolate Manufacturer's Association resulted in first action adoption of the MSRV method by AOAC International.⁵ MSRV Medium may be used as a plating medium for isolating *Salmonella* spp. (other than *S. typhi* and *S. paratyphi* type A) from stool specimens with high sensitivity and specificity.⁶

Principles of the Procedure

Enzymatic Digest of Casein and Casein Acid Hydrolysate are carbon and nitrogen sources used for general growth requirements in MSRV Medium. Sodium Chloride maintains the osmotic balance in the medium. Magnesium Chloride raises the osmotic pressure, and Potassium Dihydrogen Phosphate acts as a buffer. Malachite Green Oxalate is inhibitory to organisms other than *Salmonella* spp. Novobiocin is added as a selective agent. The low pH of the medium, combined with the presence of Malachite Green Oxalate, Magnesium Chloride, and Novobiocin, select for the highly resistant *Salmonella* spp. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	4.59 g
Casein Acid Hydrolysate	4.59 g
Sodium Chloride	7.34 g
Potassium Dihydrogen Phosphate.....	1.47 g
Magnesium Chloride, Anhydrous.....	10.93 g
Malachite Green Oxalate	0.037 g
Agar	2.7 g

Final pH: 5.6 ± 0.2 at 25°C

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

Novobiocin Supplement (7985)

Novobiocin, 20 mg

Precautions

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

Directions

1. Suspend 31.6 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. **DO NOT AUTOCLAVE.**
4. Cool medium to 45 - 50°C and aseptically add 10 ml of Novobiocin Supplement (7985).
5. Mix well and dispense into petri dishes.

Quality Control Specifications

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

Prepared Appearance: Prepared medium is trace to slightly hazy and turquoise blue.

Expected Cultural Response: Cultural response incubated aerobically at 42°C and examined for growth after 18 – 24 hours.

Microorganism	Approx. Inoculum (per drop)	Expected Results	
		Growth	Motility
<i>Citrobacter freundii</i> ATCC® 8090	> 10,000	Partial to completely inhibited	None
<i>Pseudomonas aeruginosa</i> ATCC® 27853	> 10,000	Inhibited	None
<i>Salmonella choleraesuis</i> ATCC® 13076	> 10,000	Growth	Halo
<i>Salmonella typhimurium</i> ATCC® 14028	> 10,000	Growth	Halo

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

Test Procedure^{5,7}

Pre-Enrichment: Add 25 g of cocoa or chocolate to 225 mL of sterile reconstituted nonfat dry milk with 0.45 mL of a 1% aqueous brilliant green dye solution; mix well.⁷ Incubate at 35°C for 20 ± 2 hours.

Selective Enrichment: Inoculate 10 mL of Tetrathionate Broth (prewarmed to 35°C) with 1 mL of the pre-enrichment culture. Incubate at 35°C for 8 ± 0.5 hours.

Motility Enrichment on MSRV: After selective enrichment incubation, mix the broth culture. Inoculate 3 drops at separate spots on an MSRV plate. Incubate at 42 ± 0.5°C for 16 ± 0.5 hours.

Results

Positive: Growth of migrated cells is visible as a grey-white, turbid zone extending out from the inoculated drop. Test sample is considered presumptively positive for motile *Salmonella* spp.

Negative: Medium remains blue-green around inoculation drops, with no grey-white, turbid zone extending out from the drop. Test sample is considered negative for motile *Salmonella* spp.

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 when stored as directed.

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 (MSRV) Medium Semisolid Modified	Code No.	7511A	500 g
		7511B	2 kg
		7511C	10 kg
		7985	10 mL
Novobiocin Supplement, 20 mg			

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. **DeSmedt, J. M., R. Bolderdijk, H. Rappold, and D. Lautenschlaeger.** 1986. Rapid *Salmonella* detection in food by motility enrichment on a modified semi-solid Rappaport-Vassiliadis Medium. J. Food Prot. **49**:510-514.
4. **DeSmedt, J. M., and R. Bolderdijk.** 1987. Dynamics of *Salmonella* isolation with modified semi-solid Rappaport-Vassiliadis Medium. J. Food Prot. **50**:658-661.
5. **DeSmedt, J. M., R. Bolderdijk, and J. Milas.** 1994. *Salmonella* detection in cocoa and chocolate by motility enrichment on modified semi-solid Rappaport-Vassiliadis Medium: a collaborative study. J. AOAC Int. **77**:365-373.
6. **Dusch, H., and M. Altwegg.** 1995. Evaluation of five new plating media for isolation of *Salmonella* spp. J. Clin. Micro. **33**:802-804.
7. **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.