

Rappaport-Vassiliadis (MSRV) Medium, Semisolid Modification

Novobiocin Antimicrobial Supplement

Intended Use

Rappaport-Vassiliadis (MSRV) Medium, Semisolid Modification is used with Novobiocin Antimicrobial Supplement in rapidly detecting motile *Salmonella* in feces and food products.

Summary and Explanation

MSRV Medium, Semisolid Modification is a modification of Rappaport-Vassiliadis enrichment broth for detecting motile *Salmonella* in feces and food products. The original work on MSRV medium showed that a semi-solid medium in Petri dishes could be used as a rapid and sensitive means of isolating motile *Salmonella* from food products following pre-enrichment or selective enrichment.^{1,2} The semisolid medium allows motility to be detected as halos of growth around the original point of inoculation.

The medium is recommended by the European Chocolate Manufacturer's Association. A collaborative study performed with support of the American Cocoa Research Institute and the Canadian Chocolate Manufacturer's Association resulted in first action adoption of the MSRV method by AOAC International.³

MSRV Medium, Semisolid Modification may be used as a plating medium for isolating *Salmonella* spp. (other than *Salmonella* Typhi and *Salmonella* Paratyphi A) from stool specimens with high sensitivity and specificity.^{4,5}

Principles of the Procedure

Rappaport-Vassiliadis (MSRV) Medium, Semisolid Modification contains peptones as carbon and nitrogen sources for general growth requirements. Magnesium chloride raises the osmotic pressure in the medium. Novobiocin (Novobiocin Antimicrobial Supplement) and malachite green inhibit organisms other than *Salmonella*. The low pH of the medium, combined with the novobiocin, malachite green and magnesium chloride,

helps to select for highly resistant *Salmonella* spp. Agar is the solidifying agent.

Formulae

Difco™ Rappaport-Vassiliadis (MSRV) Medium, Semisolid Modification

Approximate Formula* Per Liter	
Tryptose	4.59 g
Casein Hydrolysate (acid).....	4.59 g
Sodium Chloride	7.34 g
Monopotassium Phosphate.....	1.47 g
Magnesium Chloride (anhydrous).....	10.93 g
Malachite Green Oxalate.....	37.0 mg
Agar	2.7 g

Difco™ Novobiocin Antimicrobial Supplement

Formula per 10 mL Vial	
Sodium Novobiocin	20.0 mg

*Adjusted and/or supplemented as required to meet performance criteria.

Directions for Preparation from Dehydrated Product

Difco™ Rappaport-Vassiliadis (MSRV) Medium, Semisolid Modification

1. Suspend 31.6 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. DO NOT AUTOCLAVE. Cool to 50°C.
3. Aseptically add 10 mL of Novobiocin Antimicrobial Supplement. Mix thoroughly.
4. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Novobiocin Antimicrobial Supplement

Rehydrate with 10 mL sterile purified water. Mix well.

User Quality Control

Identity Specifications

Difco™ Rappaport-Vassiliadis Medium, Semisolid Modification

Dehydrated Appearance:	Pale green, free-flowing, homogeneous.
Solution:	3.16% solution, soluble in purified water upon boiling. Solution is blue, clear to slightly opalescent.
Prepared Appearance:	Blue, slightly opalescent, semisolid.
Reaction of 3.16% Solution at 25°C:	pH 5.2 ± 0.2

Cultural Response

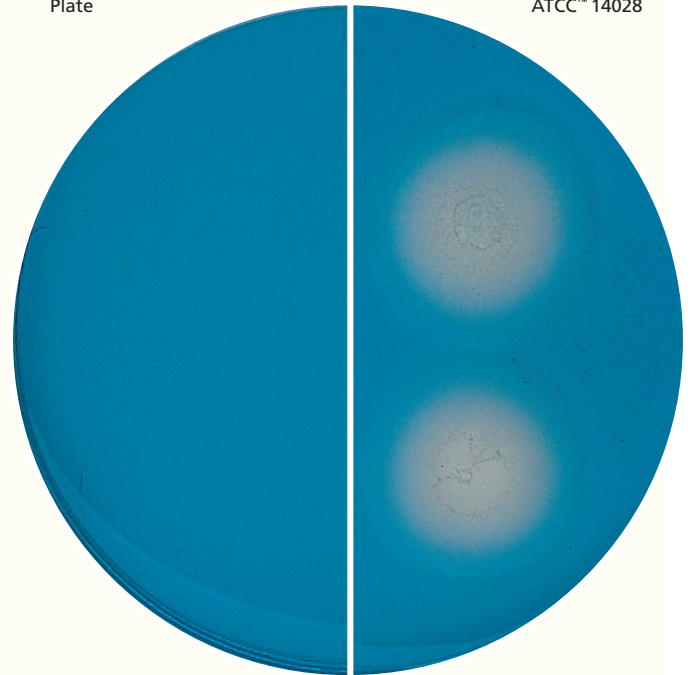
Difco™ Rappaport-Vassiliadis Medium, Semisolid Modification

Prepare the medium per label directions. Inoculate and incubate at 42 ± 0.5°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	HALO/ MOTILITY
<i>Citrobacter freundii</i>	8090	10 ³ -2 × 10 ³	Marked inhibition	–
<i>Proteus mirabilis</i>	9240	10 ³ -2 × 10 ³	None	–
<i>Pseudomonas aeruginosa</i>	27853	10 ³ -2 × 10 ³	None	–
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Enteritidis	13076	10 ² -10 ³	Good	+
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium	14028	10 ² -10 ³	Good	+
<i>Salmonella senftenberg</i> (NCTC 10384)		10 ² -10 ³	Good	+

Uninoculated Plate

Salmonella Typhimurium ATCC™ 14028



Procedure^{3,6}

Pre-enrichment

1. 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.⁶
2. Incubate at 35°C for 20 ± 2 hours.³

Selective Enrichment³

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

Motility Enrichment on MSR/V, Semisolid Modification³

5. After selective enrichment incubation, mix the broth culture. Inoculate 3 drops at separate spots on an MSR/V plate.
6. Incubate at 42 ± 0.5°C for 16 ± 0.5 hours.

Expected Results

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

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

To confirm a presumptive identification of *Salmonella*:³

Rapid serologic confirmation

1. Inoculate M Broth with growth from migration edge on MSR/V, Semisolid Modification plate.
2. Incubate at 35°C for 4-6 hours (until turbid). M Broth culture can be held for up to 24 hours at 35°C.
3. Test with *Salmonella* O and H antisera.

Culture confirmation

1. Transfer a loopful of growth from the migration edge on MSR/V, Semisolid Modification plate onto Hektoen Enteric Agar and streak for isolation.
2. Incubate at 35°C for 24 ± 2 hours.
3. From colonies of Hektoen agar that show colony appearance typical of *Salmonella* (green colonies with black centers), perform biochemical tests to confirm the identification.

Limitation of the Procedure

The combination of malachite green, magnesium chloride and a low pH may inhibit certain *Salmonella*, such as *Salmonella* Typhi and *Salmonella* Paratyphi A. Isolation techniques should include a variety of enrichment broths and isolation media.

References

1. DeSmedt, Bolderdijk, Rappold and Lautenschlaeger. 1986. J. Food Prot. 49:510.
2. DeSmedt and Bolderdijk. 1987. J. Food Prot. 50:658.
3. DeSmedt, Bolderdijk and Milas. 1994. J. AOAC Int. 77:365.
4. Dusch and Altwegg. 1995. J. Clin. Microbiol. 33:802.
5. Aspinall, Hindle and Hutchinson. 1992. Eur. J. Clin. Microbiol. Infect. Dis. 11:936.
6. Andrews and Hammack. 2001. In FDA bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.

Availability

Difco™ Rappaport-Vassiliadis (MSRV) Medium, Semisolid Modification

CCAM

Cat. No. 218681 Dehydrated – 500 g

Difco™ Novobiocin Antimicrobial Supplement

AOAC

Cat. No. 231971 Vial – 6 × 10 mL*

*Store at 2-8°C.