PRODUCT SPECIFICATION SHEET

Semisolid Rappaport Vassiliadis Medium Base, Modified (DM505)

Intended Use
Semisolid Rappaport Vassiliadis Medium Base, Modified (DM505) is recommended for detection of motile *Salmonella* species from food and environmental specimens.

Product Summary and Explanation
Semisolid Rappaport Vassiliadis Medium, Modified is a modification of Rappaport-Vassiliadis enrichment broth and prepared as per the formulation described by DeSmedt et al,[1] for detecting motile *Salmonella* in feces, food products and environmental specimens. 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. The semisolid medium allows motility to be detected as halos of growth around the original point of inoculation.[1,2] This medium detects more *Salmonella* positive samples than the routinely used enrichment procedures.[2,3,4]

Principles of the Procedure
Semisolid Rappaport Vassiliadis Medium Base, Modified contains tryptose, casein enzymic hydrolysate provide nitrogenous and carbonaceous substances and other general growth nutrients. Magnesium chloride raises the osmotic pressure in the medium. Novobiocin (Novobiocin Antimicrobic 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. Phosphate gives good buffering capacity to the medium.

Formula / Liter

Ingredients	Gms / Liter
PART A	
Tryptose	4.59
Casein enzymic hydrolysate	4.59
Sodium chloride	7.34
Potassium dihydrogen phosphate	1.47
Malachite green oxalate	0.037
Agar	2.70
PART B	
Magnesium chloride, hexahydrate	29.0
Final pH: 5.2 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

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

Directions
1. Suspend 20.72 grams of part A and 29gm of part B in 1000 ml of purified/distilled water.
2. Heat to boiling with frequent agitation to dissolve the medium completely. DO NOT AUTOCLAVE.
3. Cool to 50°C and aseptically add the rehydrated contents of 2 vials of Novobiocin Supplement (MS053).
4. Mix well and pour into sterile Petri plates.
5. Air dry the plated medium at room temperature for at least one hour.

Quality Control Specifications

Dehydrated Appearance	Light yellow to light blue homogeneous free flowing powder
Prepared Medium	Greenish blue coloured clear to slightly opalescent semisolid gel forms in Petri plates which may have a slight precipiate
Reaction of 3.16% Solution	pH : 5.2 ± 0.2 at 25°C
Gel Strength	Semisolid, comparable with 0.27% Agar gel

Expected Cultural Response: Cultural characteristics observed after an incubation at 42-43°C for 18-24 hours with added Novobiocin Supplement (MS053).

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth at 42±1°C	Motility
1.	*Citrobacter freundii ATCC 8090*	50-100	none-poor	negative reaction, no colour change
2.	*Escherichia coli ATCC 25922*	50-100	none-poor	negative reaction, no colour change
3.	*Salmonella Typhi ATCC 6539*	>=10^3	good-luxuriant	positive reaction, colourless to light pink zone
4.	*Salmonella Typhimurium ATCC 14028*	50-100	good-luxuriant	positive reaction, colourless to light pink zone
5.	*Salmonella Enteritidis ATCC 13076*	50-100	good-luxuriant	positive reaction, colourless to light pink zone

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

Test Procedure

Pre-enrichment

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

Selective Enrichment

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 MSRV, Semisolid Modification

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

Refer to appropriate references for details on test methods.

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*:

Rapid serologic confirmation

1. Inoculate M Broth with growth from migration edge on MSRV, 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 MSRV, 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.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 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 the 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.

Limitations of the Procedure

1. The combination of malachite green, magnesium chloride and a low pH may inhibit certain *Salmonella*, such as *Salmonella* Typhi and *Salmonella* Paratyphi A.
2. *Salmonella* species from faeces and subculturing on XLD Agar or Mannitol Lysine Agar results in higher recovery rates.[5]
3. This medium is not suitable for the detection of non-motile strains of *Salmonella*.[6]
4. Isolation techniques should include a variety of enrichment broths and isolation media.
5. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Semisolid Rappaport Vassiliadis Medium Base, Modified
Product Code : DM505
Available Pack sizes : 500gm

References

1. De Smedt J.M., Balderdijk R., Rappold H. and Lautenschlaeger D., 1986, J. Food Prot., 49:510.
2. De Smedt J.M., Balderdijk R., 1987, J. Food Prof., 50:658.
3. De Zutter L. et al, 1991, Int. J. Food Microbiol., 13:11.
4. De Smedt J.M. et al, 1991, Int. J. Food Microbiol., 13:301.
5. Aspinall S.T., Hindle M.A. and Hutchinson D.N., 1992, Europ. J. Clin. Microbiol. Inf. Dis., 11:936.
6. Holbrook R. et al, 1989, Lett. Appl. Microbiol., 8:139.

Further Information

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