

PRODUCT SPECIFICATION SHEET



Rappaport Vassiliadis Salmonella Enrichment Broth (DM1405H)

Intended Use

Rappaport Vassiliadis Salmonella Enrichment Broth (DM1405H) is recommended for selective enrichment of *Salmonella* species from pharmaceutical products using the microbial limit testing in compliance with the harmonized methodology of USP/EP/BP/JP.

Product Summary and Explanation

An enrichment medium for *Salmonella* that included very high amounts of malachite green and magnesium chloride as inhibitors was formulated by Rappaport et al.⁽¹⁾ The original Rappaport medium was developed for the enrichment of *S. paratyphi* and other serotypes that were known to be relatively resistant to brilliant green. In addition, magnesium chloride was found to counteract the toxic effect of the dye for *Salmonella*.⁽²⁾ Vassiliadis et al. modified the formulation by reducing the concentration of the malachite green to one third.⁽³⁾ Further, Van Schothorst and Renaud reported that using soy peptone instead of animal peptone improved recovery rates of *Salmonella*.⁽⁴⁾ Present medium is a modification of the Rappaport Vassiliadis Enrichment Broth described by Van Schothorst and Renaud.⁽⁴⁾ It is prepared in accordance with the harmonized methodology of USP/EP/BP/JP^(5,6,7,8) has been found to be superior to other *Salmonella* selective medias. By using this medium *Salmonella* species can be isolated from human faeces without pre-enrichment. Vassiliadis et al. recommended incubation of RV media at 43°C for maximum selectivity. Any deviation above 43°C may be lethal for *Salmonella*. Later, work by Peterz showed that incubation at 41.5 ± 0.5°C for 24 hours improved recovery of *Salmonella* spp.⁽⁹⁾ As compared to other bacteria, *Salmonella* generally survive at little high osmotic pressure, grow at slightly low pH and are resistant to malachite green. These characteristics of *Salmonella* are exploited in this medium for selective enrichment.

Principles of the Procedure

Rappaport Vassiliadis Soya Broth (RVS Broth) contains soya peptone that contains natural sugars which provide essential growth nutrients and enhance the growth of *Salmonella*.⁽¹⁰⁾ Magnesium chloride raises the osmotic pressure in the medium. Phosphates act as buffering agents in the medium to maintain the constant pH. Sodium chloride maintains the osmotic balance. Malachite green is inhibitory to organisms other than *Salmonellae*. The low pH of the medium, combined with the presence of malachite green and magnesium chloride, helps to select for the highly resistant *Salmonella* species. The relatively lower concentration of nutrition, also aids selective enrichment of *Salmonella*.

Formula / Liter

Ingredients	Gms / Liter
Soya peptone	4.50
Sodium chloride	8.00
Potassium dihydrogen phosphate	0.60
Dipotassium phosphate.3H ₂ O	0.40
Magnesium chloride. Hexahydrate	29.00
Malachite green	0.036
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 27.11 grams of the medium (the equivalent weight of dehydrated medium per litre) in one liter of purified/distilled water.
2. Heat if necessary to dissolve the medium completely.
3. Mix well and dispense as desired into test tubes.
4. Autoclave at 115°C / validated cycle.

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Quality Control Specifications

Dehydrated Appearance	Light yellow to light blue homogeneous free flowing powder
Prepared Medium	Greenish blue clear to slightly opalescent with a slight precipitate
Reaction of % Solution	Not Applicable
Gel Strength	Not Applicable

Cultural Response

Growth Promotion is carried out in accordance with harmonized method of USP/BP/EP/JP. Cultural response was observed after an incubation at 30-35°C for specified time. Recovery is carried out using Xylose Lysine Deoxycholate Agar (MH031), after enrichment in Rappaport Vassiliadis Salmonella Enrichment Broth.

Growth promoting properties

Clearly visible growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating ≤ 100 cfu (at 30-35°C for ≤ 18 hours).

Inhibitory properties

No growth of the test microorganism occurs for the specified temperature for not less than longest period of time specified inoculating ≥ 100 cfu (at least 100 cfu) (at 30-35°C for ≥ 24 hours).

Expected Cultural Response:

Sr. No.	Organisms	Results to be achieved					
		Inoculum (CFU)	Growth	Observed lot value (CFU)	Recovery	Colour of Colony	Incubation period
	Growth promoting	--	--	--	--	--	--
1.	<i>Salmonella Typhimurium</i> ATCC 14028	50-100	good-luxuriant	≥ 35	≥ 70 %	red with black centers	≤ 18 hrs
2.	<i>Salmonella Abony</i> NCTC 6017	50-100	good-luxuriant	≥ 35	≥ 70 %	red with black centers	≤ 18 hrs
	Inhibitory	--	--	--	--	--	--
3.	<i>Staphylococcus aureus</i> ATCC 6538	$\geq 10^3$	inhibited	0	0%	--	≥ 24 hrs
	Additional Microbiological testing	--	--	--	--	--	--
4.	<i>Escherichia coli</i> ATCC 25922	50-100	none-poor	0 -10	0 -10 %	yellow	18 -24 hrs
5.	<i>Escherichia coli</i> ATCC 8739	50-100	none-poor	0 -10	0 -10 %	yellow	18 -24 hrs
6.	<i>Salmonella Enteritidis</i> ATCC 13076	50-100	good-luxuriant	≥ 35	≥ 70 %	red with black centers	18 -24 hrs
7.	<i>Salmonella Paratyphi B</i> ATCC 8759	50-100	good-luxuriant	≥ 35	≥ 70 %	red with black centers	18 -24 hrs
8.	<i>Staphylococcus aureus</i> ATCC 25923	$\geq 10^3$	inhibited	0	0%	--	≥ 24 hrs
9.	<i>Pseudomonas aeruginosa</i> ATCC 9027	$\geq 10^3$	inhibited	0	0%	--	≥ 24 hrs
10.	<i>Pseudomonas aeruginosa</i> ATCC 27853	$\geq 10^3$	inhibited	0	0%	--	≥ 24 hrs
11.	<i>Enterococcus faecalis</i> ATCC 29212	$\geq 10^3$	inhibited	0	0%	--	≥ 24 hrs
	E. coli +S. Typhimurium (mixed culture)	--	--	--	--	--	--
12.	<i>Salmonella Typhimurium</i> ATCC 14028	50 -100	good-luxuriant	≥ 35	≥ 70 %	red with black centers	≤ 18 hrs

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

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Test Procedure

Refer to appropriate references for details on test methods.

Results

Examine selective plates for typical *Salmonella* colonies. Confirm identification of isolates by biochemical and/or serological tests as directed in appropriate references.

Storage

Store the 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 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 combined inhibitory factors of this medium (malachite green, magnesium chloride, low pH) may inhibit certain *Salmonella*, such as *S. typhi* and *S. paratyphi A*.
2. This medium was reported to be superior to *Salmonella* selective medium like Tetrathionate Broth and Selenite enrichment broth and to Tetrathionate-Brilliant Green Broth for the detection of *Salmonellae* in milk samples.
3. The enriched culture of Rappaport Vassiliadis Soya Broth (DM1405) can be further subcultured and isolated on Brilliant Green Agar (DM044) or Deoxycholate Citrate Agar (DM577), Xylose Lysine Deoxycholate Agar (DM297).
4. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Rappaport Vassiliadis *Salmonella* Enrichment Broth

Product Code : DM1405H

Available Pack sizes : 100gm / 500gm

References

1. Rappaport, Konforti and Navon. 1956. J. Clin. Pathol. 9:261.
2. Rappaport and Konforti. 1959. Appl. Microbiol. 7:63.
3. Vassiliadis, Paternaki, Papaiconomou, Papadakis and Trichopoulos. 1976. Ann. Microbiol. Inst. Pasteur. 127B:195.
4. Van Schothorst M. and Renauld A., 1983, J. Appl. Bact., 54:209.
5. The United States Pharmacopoeia, 2012, The United States Pharmacopoeial Convention. Rockville, MD.
6. British Pharmacopoeia, 2012, The Stationery office British Pharmacopoeia
7. European Pharmacopoeia, 2012, European Dept. for the quality of Medicines.
8. Japanese Pharmacopoeia, 2008.
9. Peterz M., Wiberg C. and Norberg P., 1989, J. Appl. Bact., 66:523
10. McGibbon L., Quail E. and Fricker C.R. 1984, Inter. J. Food Microbiol. 1:171

Further Information

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