



## Medium 8. Rappaport Vassiliadis Salmonella Enrichment Broth MM1491

Rappaport Vassiliadis Salmonella Enrichment Broth is recommended for selective enrichment of *Salmonella* species from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of IP.

### Composition\*\*

Ingredients	Gms / Litre
Soya peptone	4.500
Sodium chloride	8.000
Dipotassium phosphate	0.400
Potassium dihydrogen phosphate	0.600
Magnesium chloride, hexahydrate	29.000
Malachite green	0.036
pH after sterilization ( at 25°C)	5.2±0.2

\*\*Formula adjusted, standardized to suit performance parameters

### Directions

Suspend 27.11 grams of dehydrated medium (the equivalent weight of dehydrated medium per litre) in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Dispense as desired into tubes and sterilize by autoclaving at 115°C for 30 minutes or as per validated cycle.

### Principle And Interpretation

Rappaport Vassiliadis Salmonella Enrichment Medium is designed according to the revised formulation by Van Schothorst et al (1) and is recommended for the selective enrichment of Salmonellae from pharmaceutical products. This medium can also be used in direct enrichment of samples containing low inoculum. Present medium is a modification of the Rappaport Vassiliadis Enrichment Broth described by Van Schothorst and Renauld (2). It is prepared as per the formulation in Indian Pharmacopoeia (9) and is in accordance with the harmonized methodology of USP/EP/BP/JP/IP(3,4,5,6,9) has been found to be superior to other *Salmonella* selective medias. Addition of magnesium chloride to the medium was reported by Peterz et al (7). *Salmonella* species can be isolated from human faeces without pre-enrichment by using this medium.

*Salmonella* generally survive at little high osmotic pressure, grow at slightly low pH and are resistant to malachite green compared to other bacteria. These characteristics are exploited in this medium for selective enrichment of *Salmonella*. Magnesium chloride present in the medium raises the osmotic pressure. Natural sugars of soya peptone provide essential growth nutrients and enhance the growth of *Salmonella* (8). Phosphate buffers the medium to maintain constant pH. Sodium chloride maintains the osmotic balance. Malachite green inhibits many gram-positive bacteria, while selectively enriches *Salmonella*. The relatively lower concentration of nutrition, also aids selective enrichment of *Salmonella*. 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. The enriched culture of Rappaport Vasiliadis Salmonella Enrichment Broth (MM1491) can be further subcultured and isolated on Brilliant Green Agar (MM016) or Xylose Lysine Deoxycholate Agar (MM031).

### Quality Control

#### Appearance

Light yellow to light blue homogeneous free flowing powder

#### Colour and Clarity of prepared medium

Greenish blue coloured clear to slightly opalescent solution with a slight precipitate in tubes.

**pH**

5.00-5.40

**Cultural Response**

Growth Promotion is carried out in accordance with BP. Cultural response was observed after an incubation at 30-35°C for specified time. Recovery is carried out using Xylose Lysine Deoxycholate Agar (MM031) 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  $\leq 100$  cfu (at 30-35°C for  $\leq 18$  hours).

**Inhibitory properties**

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

**Cultural Response**

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of colony	Incubation temperature
<b>Growth promoting</b>						
<i>Salmonella Typhimurium</i> ATCC 14028	50 -100	luxuriant	$\geq 35$	$\geq 70$ %	red with black centers	$\leq 18$ hrs
<i>Salmonella Abony NCTC 6017</i>	50 -100	luxuriant	$\geq 35$	$\geq 70$ %	red with black centers	$\leq 18$ hrs
<b>Inhibitory</b>						
<i>Staphylococcus aureus</i> ATCC 6538	$\geq 10^3$	inhibited	0	0 %		$\geq 24$ hrs
<b>Additional Microbiological testing</b>						
<i>Escherichia coli</i> ATCC 25922	50 -100	none-poor	0 -10	0 -10 %	yellow	18 -24 hrs
<i>Escherichia coli</i> ATCC 8739	50 -100	none-poor	0 -10	0 -10 %	yellow	18 -24 hrs
<i>Salmonella Enteritidis</i> ATCC 13076	50 -100	luxuriant	$\geq 35$	$\geq 70$ %	red with black centre	18 -24 hrs
<i>Salmonella Paratyphi B</i> ATCC 8759	50 -100	luxuriant	$\geq 35$	$\geq 70$ %	red with black centre	18 -24 hrs
<i>Staphylococcus aureus</i> ATCC 25923	$\geq 10^3$	inhibited	0	0 %		$\geq 24$ hrs
<i>Pseudomonas aeruginosa</i> ATCC 9027	$\geq 10^3$	inhibited	0	0%		$\geq 24$ hrs
<i>Pseudomonas aeruginosa</i> ATCC 27853	$\geq 10^3$	inhibited	0	0 %		$\geq 24$ hrs
<i>Enterococcus faecalis</i> ATCC 29212	$\geq 10^3$	inhibited	0	0 %		$\geq 24$ hrs
<b>E.coli +S.Typhimurium (mixed culture)</b>						
<i>Salmonella Typhimurium</i> ATCC 14028	50 -100	luxuriant	$\geq 35$	$\geq 70$ %	red with black centre	18 -72 hrs

**Storage and Shelf Life**

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

**Reference**

1. Van Schothorst M., Renauld A. and VanBeek C., 1987, Food Microbiol., 4:11.
2. Van Schothorst M. and Renauld A., 1983, J. Appl. Bact., 54:209.
3. The United States Pharmacopoeia, 2011, The United States Pharmacopoeial Convention. Rockville, MD.
4. British Pharmacopoeia, 2011, The Stationery office British Pharmacopoeia
5. European Pharmacopoeia, 2011, European Dept. for the quality of Medicines.

Please refer disclaimer Overleaf.

6. Japanese Pharmacopoeia, 2008.
7. Peterz M., Wiberg C. and Norberg P., 1989, J. Appl. Bact., 66:523
8. McGibbon L., Quail E. and Fricker C.R. 1984, Inter. J. Food Microbiol . 1:171
9. Indian Pharmacopoeia, 2010 Ministry of Health and Family Welfare, Govt. of India

Revision : 1 / 2011



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