Rappaport Vassiliadis R10 Medium (DM1302)

Intended Use

Rappaport Vassiliadis R10 Medium (DM1302) is recommended for selective enrichment of *Salmonella* from meat and dairy products, fecaes and sewage polluted water.

Product Summary and Explanation

Rappaport et al.[1] formulated an enrichment medium for *Salmonella* that included malachite green and magnesium chloride as inhibitors in very high amounts. 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 neutralize the toxic effect of the dye for *Salmonella*.[2] This formula was further modified by Vassiliadis et al. in which the concentration of malachite green was reduced to one third.[3] The Rappaport formulation, designated R25/37°C, recommended incubation at 37°C; the Vassiliadis modification, designated R10/43°C, had a recommended incubation at 43°C. Peterz et al.[4] reported addition of magnesium chloride to the medium and showed that incubation at 41.5° ± 0.5°C for 24 hours improved recovery of *Salmonella* spp.[4]

Rappaport-Vassiliadis R10 Broth is a selective enrichment medium that is used following pre-enrichment of the specimen in a suitable pre-enrichment medium. It has gained approval for use in analyzing milk and milk products,[5] raw flesh foods, highly contaminated foods and animal feeds.[6]

By using this medium *Salmonella* species can be isolated from human faeces without pre-enrichment. 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 are exploited in this medium for selective enrichment of *Salmonella*. *Salmonella typhi* and *S. paratyphi A* are sensitive to malachite green and may be inhibited.

Principles of the Procedure

Rappaport Vassiliadis R10 Broth contains casein enzymic hydrolysate which provides carbon, nitrogen and other essential growth nutrients. Sodium chloride helps to maintain osmotic balance of the medium. Magnesium chloride raises the osmotic pressure in the medium. 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. Potassium phosphate buffers the medium to maintain the constant pH.

Formula / Liter

Ingredients	Gms / Liter
Casein enzymic hydrolysate	4.54
Sodium chloride	7.20
Potassium dihydrogen phosphate	1.45
Magnesium chloride	13.40
Malachite green oxalate	0.036
Final pH: 5.1 ± 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 26.62 grams in one liter of distilled water.
2. Heat, if necessary, to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.

Quality Control Specifications

Dehydrated Appearance	Light yellow to light blue homogeneous free flowing powder
Prepared Medium	Greenish blue coloured clear to slightly opalescent solution that may have precipitate.
Reaction of 2.66% solution	pH 5.1 ± 0.2 at 25°C
Gel Strength	Not Applicable

Expected Cultural Response: Cultural characteristics observed after an incubation at 42 - 43°C for 18-24 hours. After incubation, subculture on selective agar media like MacConkey Agar (DM143) or XLD Agar (DM297) and incubate at 35-37°C for 18-24 hours.

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth at 42±1°C	Recovery	Colour of Colony
1.	*Escherichia coli ATCC 25922*	50-100	none-poor	<=10%	pink-red
2.	*Salmonella Enteritidis ATCC 13076*	50-100	good-luxuriant	>=50%	colourless
3.	*Salmonella Typhi ATCC 6539*	50-100	good-luxuriant	>=50%	colourless
4.	*Salmonella Typhimurium ATCC 14028*	50-100	good-luxuriant	>=50%	colourless

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

Test Procedure

Water and Sewage Samples

For isolating *Salmonella* (other than *S. typhi*) from water and associated materials, such as sewage liquor, sewage sludge, digested sludge and pressed sludge cake:

1. Concentrate the sample by filtering it through a plug of sterile absorbent cottonwool inserted in the neck of a large sterile funnel or through a Whatman No. 17 absorbent pad.
   Pre-enrichment
2. Using aseptic technique, transfer the cottonwool plug or the pad to 100 mL of a suitable pre-enrichment medium such as Buffered Peptone Water.
3. Incubate at 37 ± 0.5°C for 18-24 hours.
   Selective Enrichment
4. Inoculate 10 mL of Rappaport-Vassiliadis R10 Broth with 0.1 mL of the pre-enrichment culture. Inoculate 10 mL of Muller-Kauffman Tetrathionate Broth with 1 mL of the pre-enrichment culture.
5. Incubate Rappaport-Vassiliadis R10 Broth at 41.5 ± 0.5°C. Incubate Muller- Kauffman Tetrathionate Broth at 42 ± 1°C for 48 hours.

Milk and Foods

For isolating *Salmonella* (other than *S. typhi*) from milk and milk products, raw flesh foods, highly contaminated foods and animal feeds:

Pre-enrichment

1. Add 25 g or a 25 mL sample of the specimen to 225 mL of pre-enrichment medium. Consult appropriate references for the type of product being tested.
2. Incubate at 35 ± 2°C for 20-24 hours or at 37°C for 16-20hours, depending on the referenced procedure being followed.
   Selective Enrichment
3. Inoculate 10 mL of Rappaport-Vassiliadis R10 Broth with 0.1 mL of pre-enrichment culture. Inoculate 10 mL of another selective enrichment medium such as Tetrathionate Broth or Selenite Cystine Broth with the recommended amount of pre-enrichment culture.
4. Incubate Rappaport-Vassiliadis R10 Broth at 41.5 ± 0.5°C for 24 ± 2 hours or at 42 ± 0.5°C for 22-24 hours. Incubate the other selective enrichment broths appropriately.

Results

Water and Sewage Samples

1. After incubation, subculture both selective enrichment broths to Brilliant Green Agar and XLD Agar. Incubate at 35 ± 2°C for 18-24 hours.
2. Examine for typical *Salmonella* colonies. Confirm identification of isolates by biochemical and serologic tests.

Milk and Foods

1. After incubation, subculture Rappaport-Vassiliadis R10 Broth and the other selective enrichment broths to selective agar media and incubate at 35 ± 2°C for 24 ± 2 hours or for 18-24 hours.
2. Examine for typical *Salmonella* colonies. Confirm identification of isolates by biochemical and serologic tests.

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 combined inhibitory factors of this medium (malachite green, magnesium chloride, low pH) may inhibit certain *Salmonella*, such as *Salmonella* Typhi and *S.* Paratyphi A. Isolation techniques should include a variety of enrichment broths and isolation media.
2. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Rappaport Vassiliadis R10 Medium
Product Code : DM1302
Available Pack sizes : 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, Papaiconomon, Papadakis and Trichopoulos. 1976. Ann. Microbiol. Inst. Pasteur. *127B*:195.
4. Peterz M., Wiberg C. and Norberg P., 1989, J. Appl. Bact., 66:523
5. International Organization for Standardization. 2001. Milk and milk products – detection of *Salmonella*. ISO 6785/IDF 93:2001. ISO, Geneva, Switzerland.
6. U.S. Department of Agriculture. Microbiology laboratory guidebook, online. Food Safety and Inspection Service, USDA, Washington, D.C.

Further Information

For further information please contact your local MICROMASTER Representative.

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