



Rappaport Vassiliadis Medium, Modified

M880F

Rappaport Vassiliadis Medium, Modified is recommended for enrichment of Salmonellae in accordance with FDA BAM, 1998

Composition**

Ingredients	Gms / 1110 ml
Tryptone	5.000
Sodium chloride	8.000
Potassium dihydrogen phosphate	1.600
Magnesium chloride hexahydrate	40.000
Malachite green oxalate	0.040
Final pH (at 25°C)	5.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 33.37 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense as desired into tubes and sterilize by autoclaving at 10 lbs pressure (115°C) for 15 minutes.

Principle And Interpretation

Rappaport Vassiliadis Medium, Modified is a modification of the formulation by Van Schothorst et al (1) and is recommended for the selective enrichment of Salmonellae from food specimens by FDA BAM, 1998(2). *Salmonella* generally survive at little high osmotic pressure, grow at slightly low pH and are resistant to malachite green compared to other bacteria. Salmonellae constitute the most taxonomically complex group of bacteria among the *Enterobacteriaceae* (3). Human *Salmonella* infections are most commonly caused by ingestion of food, water or milk contaminated by human or animal excreta. Contaminated eggs or foods containing eggs have also been a source of food borne salmonellosis.

Inoculate the samples into appropriate enriched media and incubate for 24 hrs for 24 ± 2 h at 35°C. Transfer 0.1 ml mixture to 10 ml Rappaport Vassiliadis Medium, Modified (M880F) and another 1 ml into 10 ml Tetrathionate (TT) broth (M880F). Vortex and incubate at optimum temperature for 24 ± 2 h depending upon the microbial load and type of the sample. These are further subcultured into XLD Agar or HE Agar, incubate the plates for 24 ± 2 h at 35°C and observe for the appearance of typical salmonellae colonies. Blue-green to blue colonies will be appeared in XLD Agar and pink colonies with or without black centers on HE Agar.

The medium contains Tryptone which provides essential growth nutrients. 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. Sodium chloride maintains the osmotic balance.

Quality Control

Appearance

Light yellow to light blue homogeneous free flowing powder

Colour and Clarity of prepared medium

Bluish green coloured, clear to slightly opalescent solution with slight precipitate

Reaction

Reaction of 3.34% w/v aqueous solution at 25°C. pH : 5.5±0.2

pH

5.30-5.70

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 (M081) or XLD Agar (M031) and incubate at 35-37°C for 18-24 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth at 42±1°C	Recovery	Colour of Colony
Cultural Response <i>Escherichia coli</i> ATCC 25922	50-100	none-poor	<=10%	pink-red
<i>Salmonella Enteritidis</i> ATCC 13076	50-100	good-luxuriant	>=50%	colourless
<i>Salmonella Typhi</i> ATCC 6539	50-100	good-luxuriant	>=50%	colourless
<i>Salmonella Typhimurium</i> ATCC 14028	50-100	good-luxuriant	>=50%	colourless

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.
2. FDA, U.S. 1998. Bacteriological Analytical Manual. 8 ed. Gaithersburg, MD: AOAC International.
3. Tindall, B. J., Crimont, P. A. D., Gorry, G. M. and Euzesny, B. P 2005. Int. J. Sys. Evol. Microbiol., 55.

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