



Modified Rappaport Vassiliadis Medium for Water Testing

M1658I

Modified Rappaport Vassiliadis Medium is recommended for selective enrichment of *Salmonella* species from water.

Composition**

Ingredients	Gms / Litre
Peptone from soyabeans	1.000
Peptic digest of animal tissue	4.000
Sodium chloride	8.000
Dipotassium hydrogen phosphatetrihydrate	0.400
Potassium dihydrogen phosphate	0.600
Magnesium chloride, 6H ₂ O	31.700
Malachite green	0.040
Final pH (at 25°C)	5.2±0.1

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 25.93 grams of dehydrated medium in 1000 ml distilled water. Heat gently if necessary to dissolve the medium completely. Dispense as desired into tubes and sterilize by autoclaving at 115°C for 15 minutes.

Principle And Interpretation

The original formulation described by Rappaport et al (1) with magnesium chloride hexahydrate was modified by Vassiliadis et al (2) by lowering the concentration of malachite green and raising the incubation temperature to 43°C. Modification of this formulation is recommended as the selective enrichment medium for detection of *Salmonella* from water.

The medium has animal peptones and vegetable peptones, i.e enzymatic digest of animal tissue and peptone from soyabeans which provides necessary nutrient factors. Sodium chloride maintains osmotic balance of cells. Potassium salts buffer the medium well. Divalent magnesium ions regulates cell metabolism whereas malachite green, the dye acts as a selective agent inhibiting many gram positive bacteria.

For water samples exceeding 10 ml in volume, add the sample to the same volume of Buffered Peptone Water (M614) double strength or filter through a sterile membrane filter and place the filter in 50 ml of buffered peptone water (single strength). Filtering aids can be used when needed. For sample volumes of 10 ml or less, use a minimum of 50 ml of buffered peptone water (single strength) or at least 10 times the volume of sample. Incubate at 36 ± 2° C for 16 - 20 hours.

Further transfer this pre-enriched peptone water culture, 0.1 ml to 10 ml of Modified Rappaport Vassiliadis Medium(M1658I) or 1 ml pre-enriched culture to 100 ml of M1658I. Then incubate in water bath at 42 ± 0.5°C for 18-24 hours (if an incubator other than a water bath is used, prewarm the media at this temperature before inoculation). The larger volume of inoculum might increase the probability of detecting *Salmonella* organisms. In certain cases the use of Selenite cystine medium in addition to this is recommended and further subcultured on Brilliant Green Agar(M016) or XLD(M031) agar.

Note: In order to detect slow growing *Salmonella* species, it is recommended to reinoculate solid media after continued incubation of selective liquid media for another 24 hours.

Quality Control

Appearance

Light yellow to light blue homogeneous free flowing powder

Colour and Clarity of prepared medium

Blue coloured clear solution without any precipitate

Reaction

Reaction of 2.59% w/v aqueous solution at 25°C. pH : 5.2±0.1

pH

5.10-5.30

Cultural Response

M1658I: Cultural characteristics observed after an incubation at different temperatures for 24-48 hours, when subcultured on Brilliant Green Agar Base (M016) and then incubated at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Recovery at 37°C	Recovery at 42 ± 1°C	Colour of colony
<i>Escherichia coli</i> ATCC 25922	50-100	fair	poor	yellowish green
<i>Salmonella Paratyphi B</i> ATCC 8759	50-100	good	good	pink white
<i>Salmonella Enteritidis</i> ATCC 13076	50-100	luxuriant	luxuriant	pink white
<i>Salmonella Typhi</i> ATCC 6539	50-100	fair-good	fair	pink red
<i>Salmonella Typhimurium</i> ATCC 14028	50-100	luxuriant	luxuriant	pink white

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.Rappaport F., Konforti N. and Navon B., 1956, J. Clin. Path., 9:261.
- 2.Vassiliadis P. Pateraki E., Papaiconomou N., Papadaicis J. A., Trichopoulos D., 1976, Annales de Microbiologie (Institut Pasteur), 127B : 195.

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