Rappaport Soy Broth (Vassiliadis) ISO

Cat #: MM-M-174

For the selective enrichment of Salmonella

Principles and uses:

Rappaport Soy Broth (Vassiliadis) is recommended by ISO 6579 and ISO 19250, after the preenrichment step, for the selective isolation of Salmonella spp.
Rappaport medium was modified by Vassiliadis by reducing Malachite green concentration and increasing incubation temperature, thus offering a better stability of the pH of the prepared medium and optimizing the concentration of Magnesium chloride, resulting in an improved recovery of Salmonellae.
Soy peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Potassium phosphates balance the low pH of the medium, combined with the presence of Magnesium chloride to raise the osmotic pressure, and Malachite green to inhibit other organisms. Sodium chloride supplies essential electrolytes for transport and osmotic balance.

Formula per Litre:

Dipotassium phosphate	0.18g	Magnesium chloride anhydrous	13.4g
Malaquite green	0.036g	Monopotassium phosphate	1.26g
Sodium chloride	7.2g	Soy peptone	4.5g

Preparation:

Suspend 26.6 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes and sterilize in autoclave at 121 ºC for 15 minutes.

Instructions for use:

For the detection of Salmonella spp. in food, animal feed, animal feces and environmental samples according to ISO 6579:

- Pre-enrichment in non-selective liquid medium:

Inoculate Buffered Peptone Water (Cat. MM-M- 402) with the sample or dilutions, and incubate at 34-38 °C for 18 h.

- Enrichment in/on selective media:

Inoculate, with the culture obtained in the pre-enrichment stage the Rappaport Soya Broth (Vassiliadis) (Cat. MM-M-174) or Rappaport Semi-Solid Medium Vassiliadis Modified (MSRV) (Cat. MM-M-376), and Tetrathionate Broth (Muller-Kauffmann) (Cat. MM-M-173).
The Soy Rappaport Broth and the Modified Rappaport Semisolid Medium are incubated at 41.5 °C for 24 h, and the Tetraetium Broth at 37 °C for 24 h.

- Plating in selective solid media:

From the selectively enriched cultures, inoculate two selective isolates mediums; XLD agar (Cat. MM-M-274) and any other selective media complementary to XLD agar (Chromogenic Salmonella Agar (Cat. MM-M-122)), Bright Green Agar (Cat. MM-M-143), Bismuth Sulfite Agar (Cat. MM-M-011), DCLS agar (Cat. MM-M-045), Citrate Deoxycholate Agar (Cat. MM-M-067), Enteric Hektoen Agar (Cat. MM-M-030), Salmonella Shigella Agar (Cat. MM-M-064) and XLT4 Agar (Cat. MM-M-159).
Incubate inverted XLD plates at 37 °C for 24 ± 3 h.
Incubate the second selective medium according to the manufacturer's instructions.

- Confirmation:

Subculture presumptive colonies of Salmonella and confirm their identity through biochemical and serological tests.
For the detection of Salmonella spp. in water samples according to ISO 19250:

- Pre-enrichment in a non-selective medium:

Inoculate Buffered Peptone Water (Cat. MM-M-402) with the sample or dilutions, and incubate at 36±2 °C for 18±2 h.

- Enrichment in selective media:

Inoculate, with the culture obtained in the pre-enrichment stage, the Rapport Soy Broth (Vassiliadis) (Cat. MM-M-174) and the Tetrathionate Broth (Muller-Kauffmann) (Cat. MM-M-173).
The Rapport Soy Broth is incubated at 41.5±1 °C and the Tetraethionate broth at 37±1 °C, both for 24±3 h.

- Plating in selective solid media:

From the selectively enriched cultures, inoculate two selective isolation agar; XLD Agar (Cat. MM-M-274) and any other selective media complementary to XLD agar (For example, Bright Green Agar (Cat. MM-M-143) or Bismuth Sulfite Agar (Cat. MM-M-011).
Incubate inverted XLD plates at 36±2 °C for 24±3 h.
Incubate the second selective medium according to the manufacturer's instructions.

- Confirmation:

Subculture presumptive colonies of Salmonella and confirm their identity through biochemical and serological tests.

Quality control:

Solubility	Appearance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Beige	Blue	5.2 ± 0.2

Microbiological test:

According to ISO 11133:

Incubation conditions: (41.5±1ºC /24±3 h).
Inoculation conditions: Productivity qualitative (<100 CFU) / Selectivity (10^4-10^6 CFU).

Microorganisms	Specification	Characteristic reaction
Salmonella typhimurium ATCC 14028 +Escherichia coli ATCC 8739 +Pseudomonas aeruginosa ATCC 27853	>10 colonies in XLD or other chosen medium	Colonies with black centre and a lightly transparent zone of reddish color due to the color change of the medium
Salmonella enteriditis ATCC 13076 +Escherichia coli ATCC 8739 +Pseudomonas aeruginosa ATCC 27853	>10 colonies in XLD or other chosen medium	Colonies with black centre and a lightly transparent zone of reddish color due to the color change of the medium
Enterococcus faecalis ATCC 29212	<10 colonies in TSA	
Escherichia coli ATCC 8739	Partial incubation <100 colonies in TSA	

Storage:

Temperature: 2ºC - 25ºC

Bibliography:

Rappaport F., Konforti N. and Navon B. (1 956) J. Clin Pathol., 9,261. Peterz M. Wiberg C. and Norberg P. (1989) J. Appl. Bact. 66: 523-528.
UNE-EN-ISO 6579. Food Microbiology for human consumption and Animal Feed. Horizontal Method for the detection of Salmonella spp. ISO19250 Water quality-Detection of Salmonella spp.