

Rappaport Soy Broth (Vassiliadis) ISO

Cat. 1174

For the selective enrichment of Salmonella

Practical information

Applications	Categories
Selective enrichment	Salmonella

Industry: Water / Food

Regulations: ISO 11133 / ISO 19250 / ISO 6579

Principles and uses

Rappaport Soy Broth (Vassiliadis) is recommended by ISO 6579 and ISO 19250, after the pre-enrichment 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 in g/L

Dipotassium phosphate	0,18	Magnesium chloride anhydrous	13,4
Malaquite green	0,036	Monopotassium phosphate	1,26
Sodium chloride	7,2	Soy peptone	4,5

Preparation

Suspend 26,6 grams of the medium in 1 liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 115 °C for 15 minutes. DO NOT OVERHEAT.

Instructions for use

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

- Preenrichment in non-selective liquid medium:

Inoculate the Buffered Peptone Water (Cat. 1402) with the sample or dilutions, and incubate at 34-38 °C for 18±2 h.

- Enrichment in/on selective media:

Inoculate, with the culture obtained in the pre-enrichment stage, the Rappaport Soy Broth (Vassiliadis)(Cat. 1174) or the Modified Semisolid Rappaport Vassiliadis medium (MSRV) (Cat. 1376), and the MKKTN Broth(Cat. 1173).

The Rappaport Soy Broth and the Modified Semisolid Rappaport medium are incubated at 41,5 °C for 24±3 h, and the MKKTN Broth at 34-38 °C for 24±3 h.

- Plating out on selective solid media:

From the selective enriched cultures, inoculate two selective isolation agar; XLD agar (Cat. 1274) and any other selective medium complementary to XLD agar (Salmonella Chromogenic Agar (Cat. 1122), Brilliant Green Agar (Cat. 1143), Bismuth Sulfite Agar (Cat. 1011), DCLS Agar(Cat. 1045), Desoxycholate Citrate Agar (Cat. 1067), Hektoen Enteric Agar (Cat. 1030), Salmonella Shigella Agar(Cat. 1064) and XLT4 Agar (Cat. 1159)).

Incubate the XLD plates inverted at 34-38 °C for 24±3 h.

Incubate the second selective medium in accordance with the manufacturer's instructions.

- Confirmation:

Subculture colonies of presumptive Salmonella and confirm their identity by biochemicals and serological tests.

Note: According to Annex D of ISO 6579-1: 2017, for the detection of enterica subspecies enterica serovars Typhi and Paratyphi, Selenite Cystine Broth (Cat. 1220) should be added as a selective enrichment medium and Bismuth Sulfite Agar (Wilson Blair) should be selected as a second selective medium (Cat. 1011).

* For detection of Salmonella spp. in water samples according to ISO 19250:

- Preenrichment in non-selective medium:

Inoculate the Buffered Peptone Water (Cat. 1402) with the sample or dilutions, and incubate at 34-38 °C for 18±2 h.

- Enrichment in selective media:

Inoculate, with the culture obtained in the pre-enrichment stage, the Rappaport Soy Broth (Vassiliadis)(Cat. 1174) and the MKKTN Broth (Cat. 1173). The Rappaport Soy Broth is incubated at 41,5±1 °C and the MKKTN Broth at 34-38 °C, both of them for 24±3 hours.

- Plating out on selective solid media:

From the selective enriched cultures, inoculate two selective isolation agar; XLD agar (Cat. 1274) and any other selective medium complementary to XLD agar (For instance, Brilliant Green Agar (Cat. 1143) or Bismuth Sulfite Agar (Cat. 1011))

Incubate the XLD plates inverted at 34-38 °C for 24±3 hours.

Incubate the second selective medium in accordance with the manufacturer's instructions.

- Confirmation:

Subculture colonies of presumptive Salmonella and confirm their identity by biochemicals and serological tests.

Quality control

Solubility	Appearance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25°C)
w/o rests	Fine powder	Beige	Blue	5,2±0,2

Microbiological test

According to ISO 11133:

Incubation conditions: (41,5±1 °C / 24±3 h).

Inoculation conditions: Productivity qualitative (<100 CFU) / Selectivity (10⁴-10⁶ 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 enteritidis 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 inhibition <100 colonies in TSA	

Storage

Temp. Min.:2 °C

Temp. Max.:25 °C

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.

ISO 6579 Microbiology of food and animal feeding stuffs – Horizontal method for the detection of Salmonella spp. Amendment 1: Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSRV and SC.