Rappaport Vassiliadis Soyabean Meal Broth

M1448I

Intended use

Recommended as selective enrichment medium for the isolation of *Salmonella* species. The composition and performance criteria of this medium are as per the specifications laid down in ISO 6579-1:2017, Amd. 2020/ ISO 19250:2010 and ISO 11133:2014 (E) /Amd. :2020.

Composition**

ISO Specification - RVS Broth

Ingredients	g / L
Enzymatic digest of soya Sodium	4.500
chloride	7.200
Potassium dihydrogen phosphate	1.440
($KH_2PO4 + K_2HPO_4$)	
Magnesium chloride, hexahydrate	28.600
Malachite green , oxalate	0.036
Final pH (after sterilization)	5.2±0.2

Rappaport Vassiliadis Soyabean Meal Broth

Ingredients	g / L
Soya peptone#	4.500
Sodium chloride	7.200
Potassium dihydrogen phosphate	1.260
Dipotassium hydrogen phosphate	0.180
Magnesium chloride, hexahydrate	28.600
Malachite green, oxalate	0.036
Final pH (after sterilization)	5.2±0.2

- Equivalent to enzymatic digest of soya
**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 26.58 gram (the equivalent weight of dehydrated medium per litre) in 1000 ml purified/distilled water. Heat gently if necessary to dissolve the medium completely. Dispense into tubes or flasks as desired and sterilize by autoclaving at 115°C for 15 minutes.

Principle And Interpretation

Rappaport Vassiliadis Soyabean Meal Broth (RVSM) is modification of the Rappaport Vassiliadis Enrichment Broth, revised by van Schothorst (1,2,3). This medium is recommended as the selective enrichment medium for isolation of *Salmonella*. van Schothorst modified the original formula by addition of dipotassium hydrogen phosphate to buffer the medium and addition of anhydrous magnesium chloride to enhance the reliability of enrichment broth. Peterz (4) et al have also emphasized the importance of the concentration of magnesium chloride in the final medium. This medium is recommended by ISO for food samples (5) and water samples (6).

The medium contains soya peptone 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. Phosphates buffer the medium to maintain the constant pH. Sodium chloride maintains the osmotic balance.

Type of specimen

Food, milk and millk products, animal feed, environmental samples

Specimen Collection and Handling

Processesing : (5, 6,7)

Pre-enrichment : Samples (25 gram in 225 ml) are preenriched in Buffered Peptone Water (M1494I) and incubated at 34°C to 38°C for 18 h ± 2 hours.

Selective enrichment: 0.1 ml of pre- enriched sample is inoculated in 10 ml RVS Broth (M1448I) incubated at 41.5 ± 1°C for 24 ± 3 hours.

Isolation : The culture thus obtained is then plated on XLD Agar (M031I) and second plating media Bismuth Sulphite Agar (BS) (M027I) and incubated at 37± 1°C for 24 ± 3 hours. An additional incubation of 24 ± 3 hours is recommended in case of Bismuth Sulphite Agar.

Confirmation : Biochemical and serological tests are performed for confirmation.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets

Limitations :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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 2.66% w/v aqueous solution at 25°C. pH : 5.2±0.2

pH

5.00-5.40

Cultural Response

Productivity : Cultural characteristics observed after an incubation at 41.5°C ± 1°C for 24±3 h. Further subculture is carried out on XLD Agar, Modified (M031I) and incubated at 37±1°C for 24±3 hours.

Selectivity : Cultural characteristics observed after an incubation at 41.5°C ± 1°C for 24±3 h. Further subculture is carried on TSA at 37±1°C for 24±3 hours.

Organism	Inoculum (CFU)	Recovery on XLD Agar (M031I)	Colour of colony on XLD Agar (M031I)
Productivity			
Salmonella Enteritidis ATCC 13076 (00030*)+ *Esccherichia coli* ATCC 8739 (00012*) + *Pseudomonas aeruginosa* ATCC 27853 (00025*)	50-100 >=10^4 >=10^4	>10 colonies	red colonies w/ black centre
Salmonella Typhimurium ATCC 14028 (00031*)+ *Esccherichia coli* ATCC 25922 (00013*) + *Pseudomonas aeruginosa* ATCC 27853 (00025*)	50-100 >=10^4 >=10^4	>10 colonies	red colonies w/ black centre
Selectivity			
Escherichia coli ATCC 8739 (00012*)	>=10^4	partial inhibition	<=100 colonies on Tryptone Soya Agar
Escherichia coli ATCC 25922 (00013*)	>=10^4	partial inhibition	<=100 colonies on Tryptone Soya Agar
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibition - partial inhibition	<10 colonies on Tryptone Soya Agar
Enterococcus faecalis ATCC 19433 (00009*)	>=10^4	inhibition - partial inhibition	<10 colonies on Tryptone Soya Agar

Key : *- Corresponding WDCM Numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

Reference

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4. Peterz M., Wiberg C. and Norberg P., 1989, J. Appl. Bacteriol., 66,523-528.
5. Microbiobiology of the food chain- Horizontal method for the detection, enumeration and serotyping of *Salmonella*- Part I Detection of *Salmonella* . International Organization for Standardization (ISO), ISO/DIS 6579-1:2017.
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