Rappaport Vassiliadis Salmonella Enrichment Broth

LQ104

Intended use

For selective enrichment of *Salmonella* species from pharmaceutical, food samples in accordance with harmonized methods of USP, EP, BP, IP & JP. It can also be used for clinical samples.

Composition**

Ingredients	g / L
Soya peptone	4.500
Sodium chloride	8.000
Dipotassium hydrogen phosphate	0.400
Potassium dihydrogen phosphate	0.600
Magnesium chloride, hexahydrate	29.000
Malachite green	0.036

**Formula adjusted, standardized to suit performance parameters

Directions

Label the ready to use LQ104 bottle. Inoculate the sample and Incubate at specified temperature and time.

Principle And Interpretation

Rappaport Vassiliadis Salmonella Enrichment Medium is designed according to the revised formulation by Van Schothorst et al (1) and is recommended for the selective enrichment of Salmonellae from pharmaceutical products. This medium can also be used in direct enrichment of samples containing low inoculum. Present medium is a modification of the Rappaport Vassiliadis Enrichment Broth described by Van Schothorst and Renauld (2). It is prepared in accordance with the harmonized methodology of USP/EP/BP/JP/IP (3,4,5,6,7) has been found to be superior to other *Salmonella* selective medias. Addition of magnesium chloride to the medium was reported by Peterz et al (8). *Salmonella* species can be isolated from human faeces without pre-enrichment by using this medium.

Salmonella generally survive at little high osmotic pressure, grow at slightly low pH and are resistant to malachite green compared to other bacteria. These characteristics are exploited in this medium for selective enrichment of *Salmonella.* Magnesium chloride present in the medium raises the osmotic pressure. Natural sugars of soya peptone provide essential growth nutrients and enhance the growth of *Salmonella* (9). Phosphate buffers the medium to maintain constant pH. Sodium chloride maintains the osmotic balance. Malachite green inhibits many gram-positive bacteria, while selectively enriches *Salmonella.*

The relatively lower concentration of nutrition, also aids selective enrichment of *Salmonella.* This medium was reported to be superior to *Salmonella* selective medium like Tetrathionate Broth and Selenite enrichment broth and to Tetrathionate-Brilliant Green Broth for the detection of Salmonellae in milk samples. The enriched culture of Rappaport Vasiliadis Salmonella Enrichment Broth (LQ104) can be further subcultured and isolated on Xylose Lysine Deoxycholate Agar (MPH031).

Type of specimen

Pharmaceutical samples; Clinical samples: faeces, preferably faecal dilutions, Food samples

Specimen Collection and Handling

For pharmaceutical samples follow appropriate techniques for handling specimens as per established guidelines (3,4,5,6,7).

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (10,11).

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (9).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic Use. For professional use only. 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 clinical 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
Sterile clear Rappaport Vassiliadis Salmonella Enrichment Broth in tubes.
Colour
Bluish green coloured solution.
Quantity of medium
10 ml of medium in tubes.
Reaction
5.00- 5.40
Sterilization Method
Sterilized by autoclaving at 115 °C as per validated cycle
Sterility Assurance Level
Sterility assurance level of media was validated against *B.subtilis* Spore strips. The spore strips exposed at 115°C and unexposed strips were inoculated separately in 100 ml Soyabean Casein Digest Medium and incubated at 35°C for 7 days.
Exposed spore strips
No growth observed
Unexposed spore strips
Luxuriant growth observed
Sterility Check
Passes release criteria
Cultural Response
Cultural characteristics observed after incubation at 30-35°C for 18-24 hours. Recovery is carried out using XLD Agar (MH031/MPH031).
Growth promoting properties
Clearly visible growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating <=100 cfu (at 30-35°C for <=18 hours).
Inhibitory properties
No growth of the test microorganism occurs for the specified temperature for not less than longest period of time specified inoculating >=100 cfu (at least 100 cfu) (at 30-35°C for >= 24 hours).

Organism	Inoculum (CFU)	Growth	Colour of colony	Incubation Period
Growth promoting				
Salmonella Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant	red with black centers	<=18 hrs
Salmonella Abony NCTC 6017 (00029*)	50 -100	luxuriant	red with black centers	<=18 hrs
Inhibitory				
Staphylococcus aureus subsp. aureus ATCC 6538 (00032*)	>=10^3	inhibited		>=24 hrs
Additional Microbiological testing				
Escherichia coli ATCC 25922 (00013*)	50 -100	none-poor	yellow	18 -24 hrs
Escherichia coli ATCC 8739 (00012*)	50 -100	none-poor	yellow	18 -24 hrs
Salmonella Enteritidis ATCC 13076 (00030*)	50 -100	luxuriant	red with black centre	18 -24 hrs

Salmonella Paratyphi B ATCC 8759	50 -100	luxuriant	red with black centre	18 -24 hrs
*Staphylococcus aureus subsp. aureus*ATCC 25923 (00034*)	>=10^3	inhibited		>=24 hrs
^*Pseudomonas paraeruginosa ATCC 9027* (00026*)	>=10^3	inhibited		>=24 hrs
Pseudomonas aeruginosa ATCC 27853(00025*)	>=10^3	inhibited		>=24 hrs
Enterococcus feacalis ATCC 29212 (00087*)	>=10^3	inhibited		>=24 hrs
E.coli +S.Typhimurium (mixed culture)				
Salmonella Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant	red with black centre	18 -72 hrs

Key : (*) Corresponding WDCM numbers. ^ Formerly known as *Pseudomonas aeruginosa*

Storage and Shelf Life

Store between 15-30°C. Use before expiry date on the label. 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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (10,11).

Reference

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IVD *In vitro* diagnostic medical device

30°C / 15°C Storage temperature

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CE CE Marking

Do not use if package is damaged