

Technical Information

Rappaport Vassiliadis Salmonella Enrichment Broth

Product Code: DM 2491H

Application: - Rappaport Vassiliadis Salmonella Enrichment Broth is used for selective enrichment of *Salmonella* species from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP.

Composition**

Ingredients	Gms / Litre
Soya peptone	4.500
Sodium chloride	8.000
Dipotassium hydrogen phosphate	0.400
Potassium dihydrogen phosphate	0.600
Magnesium chloride, hexahydrate	15.4
Malachite green	0.036
pH after sterilization (at 25°C)	5.2±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Rappaport Vassiliadis Salmonella Enrichment Medium is designed according to the revised formulation by Van Schothorst et al (10) 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 (11). It is prepared in accordance with the harmonized methodology of USP/EP/BP/JP/IP (9,1,2,5,3) 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 supply essential growth nutrients and enhance the growth of *Salmonella* (4). Phosphate buffers the medium to maintain constant pH. Sodium chloride helps to maintain 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 Vassiliadis Salmonella Enrichment Broth (DM2491H) can be further subcultured and isolated on Xylose Lysine Deoxycholate Agar (DM 1031H).

Type of specimen

Pharmaceutical samples

Specimen Collection and Handling:

For pharmaceutical samples follow appropriate techniques for handling specimens as per established guidelines (9,1,2,5,3). After use, contaminated materials must be sterilized by autoclaving before discarding.



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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

Overheating may destroy the selectivity of medium.

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.

Methodology

Suspend 27.11 grams of dehydrated powder media in 1000 ml distilled water. Mix thoroughly & heat if necessary to dissolve the medium completely. Dispense as desired into tubes and sterilize by autoclaving at 115°C as per validated cycle.

Quality Control

Appearance

Light yellow to light blue homogeneous free flowing powder

Colour and Clarity

Greenish blue coloured clear to slightly opalescent solution with a slight precipitate in tubes.

pH Range

5.00-5.40

Cultural Response

Growth Promotion is carried out in accordance with harmonized method of USP/BP/EP/JP/IP. Cultural response was observed after an incubation at 30-35°C for specified time. Recovery is carried out using Xylose Lysine Deoxycholate Agar (DM 1031H), after enrichment in Rappaport Vassiliadis Salmonella Enrichment Broth.

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	Observed Lot value (CFU)	Recovery	Colour of colony	Incubation temperature
Growth promoting						
<i>Salmonella Typhimurium</i> ATCC 14028 (00031*)	50 -100	luxuriant	≥ 35	≥ 70 %	red with black centers	≤ 18 hrs
<i>Salmonella Abony</i> NCTC 6017 (00029*)	50 -100	luxuriant	≥ 35	≥ 70 %	red with black centers	≤ 18 hrs
Inhibitory						
<i>Staphylococcus aureus</i> ATCC 6538 (00032*)	$\geq 10^3$	inhibited	0	0 %	-	≥ 24 hrs
Additional Microbiological testing						
<i>Escherichia coli</i> ATCC 25922 (00013*)	50 -100	none-poor	0 -10	0 -10 %	yellow	18 -24 hrs
<i>Escherichia coli</i> ATCC 8739 (00012*)	50 -100	none-poor	0 -10	0 -10 %	yellow	18 -24 hrs





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<i>Salmonella Enteritidis</i> ATCC 13076 (00030*)	50 -100	luxuriant	>=35	>=70 %	red with black centre	18 -24 hrs
<i>Salmonella Paratyphi B</i> ATCC 8759	50 -100	luxuriant	>=35	>=70 %	red with black centre	18 -24 hrs
<i>Staphylococcus aureus</i> subsp. <i>Aureus</i> ATCC 25923 (00034*)	>=10 ³	inhibited	0	0 %	-	>=24 hrs
<i>Pseudomonas aeruginosa</i> ATCC 9027 (00026*)	>=10 ³	inhibited	0	0%	-	>=24 hrs
<i>Pseudomonas aeruginosa</i> ATCC 27853 (00025*)	>=10 ³	inhibited	0	0%	-	>=24 hrs
<i>Enterococcus faecalis</i> ATCC 29212 (00087*)	>=10 ³	inhibited	0	0%	-	>=24 hrs
E.coli +S.Typhimurium (mixed culture)						
<i>Salmonella Typhimurium</i> ATCC 14028 (00031*)	50 -100	luxuriant	>=35	>=70 %	red with black centre	18 -72 hrs

Key: (*) Corresponding WDCM numbers.

Storage and Shelf Life

Dried Media: Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°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. 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 (4, 6).

Further Reading

1. British Pharmacopoeia, 2016, The Stationery office British Pharmacopoeia
2. European Pharmacopoeia, 2017, European Dept. for the quality of Medicines.
3. Indian Pharmacopoeia, 2018, Govt. of India, Ministry of Health and Family Welfare, New Delhi
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
5. Japanese Pharmacopoeia, 2016.
6. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock, D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
7. McGibbon L., Quail E. and Fricker C.R. 1984, Inter. J. Food Microbiol. 1:171
8. Peterz M., Wiberg C. and Norberg P., 1989, J. Appl. Bact., 66:523
9. The United States Pharmacopoeia, 2019 The United States Pharmacopoeial Convention. Rockville, MD.
10. Van Schothorst M., Renaud A. and VanBeek C., 1987, Food Microbiol., 4:1
11. Van Schothorst M. and Renaud A., 1983, J. Appl. Bact., 54:20





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Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
- The product conform solely to the technical information provided in this booklet and to the best of knowledge research and development work carried at CDH is true and accurate
- Central Drug House Pvt. Ltd. reserves the right to make changes to specifications and information related to the products at any time.
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