

Rappaport Vassiliadis Soya HiCynth™ Broth (RVS HiCynth™ Broth)

MCD1491

Intended Use

Recommended as a selective enrichment medium for the Salmonellae species from the food and animal feeding stuffs.

Composition**

Ingredients	Gms / Litre
HiCynth™ Peptone No.4*	4.500
Sodium chloride	8.000
Potassium dihydrogen phosphate	0.600
Dipotassium hydrogen phosphate	0.400
Magnesium chloride hexahydrate	29.000
Malachite green	0.036
Final pH (at 25°C)	5.2±0.2

**Formula adjusted, standardized to suit performance parameters

* Chemically defined peptone

Directions

Suspend 27.11 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Dispense into tubes or flasks as desired. Sterilize by autoclaving at $\Delta 115^{\circ}\text{C}$ for 15 mins. *Note: Δ Corresponds to 10 lbs pressure.*

Principle And Interpretation

Rappaport Vassiliadis Soya Broth 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. Rappaport Vassiliadis Soya HiCynth™ Broth is prepared by replacing animal and vegetable peptones with chemically defined peptones to avoid BSE/TSE risks associated with animal peptones. 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). Addition of magnesium chloride to the medium was reported by Peterz et al (3). *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. HiCynth™ Peptone No. 4 provides carbon, nitrogen compounds, vitamins and other growth nutrients to enhance the growth of *Salmonella* (5). Phosphate buffers the medium to maintain constant pH. Sodium chloride maintains the osmotic balance. Malachite green inhibits many gram-positive bacteria, while selectively enrich *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 *Salmonella* in milk samples. The enriched culture of Rappaport Vassiliadis Soya HiCynth™ Broth (MCD1491) can be further subcultured and isolated on Brilliant Green HiCynth™ Agar (MCD016) or Deoxycholate Citrate HiCynth™ Agar (MCD065), Xylose Lysine Deoxycholate HiCynth™ Agar (MCD031).

Type of specimen

Food samples and animal feeding stuffs.

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (5).

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

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. This medium contains inhibitory substances and may not support the growth of certain *Salmonella* species like *S. Typhi*.
2. Less selective enrichment broth must be used in conjunction.
3. After enrichment the organisms must be isolated on less selective media along with selective media.
4. Further biochemical and serological testing must be carried out for confirmation.

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

Greenish blue clear to slightly opalescent with a slight precipitate.

Reaction

Reaction of 2.77% w/v aqueous solution at 25°C. pH : 5.2±0.2

pH

5.00-5.40

Cultural Response

Cultural response was observed after an incubation at 30-35°C for 18-24 hours Recovery is carried out using Xylose Lysine Deoxycholate Agar (MCD031) after enrichment.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
<i>E.coli</i> + <i>S.Typhimurium</i> (mixed culture)				
<i>E.coli</i>	50 -100	none-poor	<=10 %	yellow
<i>S.Typhimurium</i>	50 -100	luxuriant	>=50 %	red with black centers
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	>=10 ⁴	inhibited	0%	
<i>Enterococcus faecalis</i> ATCC 29212 (00087*)	>=10 ⁴	inhibited	0%	
<i>Salmonella</i> Abony NCTC 6017 (00029*)	50-100	luxuriant	>=70 %	red with black centers
<i>Salmonella</i> Typhimurium subsp. <i>aureus</i> ATCC 14028 (00031*)	50-100	luxuriant	>=70 %	red with black centers
<i>Staphylococcus aureus</i> ATCC 6538 (00032*)	>=10 ⁴	inhibited	0%	
<i>Escherichia coli</i> ATCC 25922 (00013*)	50 -100	none-poor	0 -10	yellow
<i>Salmonella</i> Enteritidis ATCC 13076 (00030*)	50-100	luxuriant	>=70 %	red with black centre
<i>Escherichia coli</i> ATCC 8739 (00012*)	50-100	none-poor	<=10 %	yellow
<i>Salmonella</i> Paratyphi B ATCC 8759	50-100	luxuriant	>=70 %	red with black centre

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store below 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 (6,7).

Reference

1. Van Schothorst M., Renauld A. and VanBeek C., 1987, Food Microbiol., 4:11.
2. Van Schothorst M. and Renauld A., 1983, J. Appl. Bact., 54:209.
3. Peterz M., Wiberg C. and Norberg P., 1989, J. Appl. Bact., 66:523
4. McGibbon L., Quail E. and Fricker C.R. 1984, Inter. J. Food Microbiol. 1:171.
5. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
7. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 02/2023

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.