

Salmonella Differential HiVeg[®] Agar (Twin pack) (RajHans HiVeg[®] Medium)

MV1078

Intended Use:

Recommended for identification and differentiation of *Salmonella* species from members of *Enterobacteriaceae*, especially *Proteus* species.

Composition**

Ingredients	g / L
Part A	-
HiVeg [®] special peptone	8.000
Yeast extract	2.000
Synthetic detergent No. III	1.000
B. C. Indicator	2.000
Agar	12.000
Part B	-
Propylene glycol	10.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 10 grams of fluid Part B in 1000 ml purified / distilled water. Add 25 grams of Part A. Mix well and heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Salmonella Differential Agar is slight modification of original formulation of Rambach (1) used for differentiation of *Salmonella* species from *Proteus* species and other enteric bacteria. Production of acid from propylene glycol is a novel characteristic of *Salmonella* species and is utilized in these media. Many of the media such as SS Agar, XLD Agar recommended for the identification and differentiation of *Salmonella* species (2) are based on lactose fermentation and hydrogen sulphide production. Salmonella Differential HiVeg[®] Agar is prepared by completely replacing animal based peptone with vegetable peptones to avoid BSE/TSE risks associate with animal peptones.

HiVeg[®] special peptone and yeast extract provides carbonaceous, nitrogenous compounds, long chain amino acids, vitamins and other growth factors supports the luxuriant growth of bacteria. Synthetic detergent No. III inhibits gram-positive organisms rendering the medium selective for enteric microorganisms. The BC indicator turns pink in presence of acid produced from propylene glycol. Lactose fermenting ability is determined by using an indicator, which can detect the presence of enzyme β -galactosidase. Lactose fermenting (β -galactosidase producing) bacteria yield blue violet coloured colony (3). *Salmonellae* produce acid from propylene glycol and on combining with the pH indicator gives typical pink red colonies. Other enteric gram-negative bacteria form colourless colonies.

Salmonella Typhimurium and *Salmonella* Enteritidis produce pink to red colonies. Specimen should be enriched in an appropriate selective enrichment broth. This enriched culture is then inoculated on Salmonella Differential Agar and incubated at 35-37°C for 24-48 hours.

Type of specimen

Water samples and Food samples

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (4).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (2).

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. The medium is selective for *Salmonella* and may not support the growth of other microorganisms.
2. Most of the *Salmonella* strains shows pink-red colonies except few which may show colourless colonies.
3. Due to nutritional variations, some strains may show poor growth.
4. Final confirmation of suspected colonies must be carried out by serological and biochemical tests.

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

Part A : Light yellow to light pink homogeneous free flowing powder Part B: Colourless viscous solution

Gelling

Firm, comparable with 1.2% Agar gel.

Colour and Clarity of Prepared medium

Light orange coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 2.5% w/v aqueous solution of Part A at 25°C. pH : 7.3±0.2

pH

7.10-7.50

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
<i>Proteus mirabilis</i> ATCC 25933	50-100	luxuriant	≥50%	colourless
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	50-100	luxuriant	≥50%	pink-red
<i>Salmonella</i> Enteritidis ATCC 13076 (00030*)	50-100	luxuriant	≥50%	pink-red
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	luxuriant	≥50%	blue-green
<i>Klebsiella pneumoniae</i> ATCC 13883 (00097*)	50-100	luxuriant	≥50%	blue-violet
<i>Salmonella</i> Typhi ATCC 6539	50-100	luxuriant	≥50%	colourless
<i>Shigella flexneri</i> ATCC 12022 (00126*)	50-100	luxuriant	≥50%	colourless
<i>Staphylococcus aureus</i> Subsp. <i>aureus</i> ATCC 25923 (00034*)	≥10 ⁴	inhibited	0%	

Key : (*) Corresponding WDCM numbers

Storage and Shelf Life

Store between 15-25°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. 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 (5,6).

Reference

1. Rambach A., 1990, Appl Environ. Microbiol., 56:301.
2. Lipps WC, Braun-Howland EB, Baxter TE, eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
3. Greenwald R., Henderson R.W. and Yappaw S., 1991, J. Clin. Microbiol. 29:2354.
4. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
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.

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Disclaimer :

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