

Deoxycholate Citrate Agar, HiVeg[®]

MV065

Intended Use:

Recommended as a selective medium for isolation of enteric pathogens particularly *Salmonella* and *Shigella* species from various specimens.

Composition**

Ingredients	g / L
HiVeg [®] infusion	10.000
HiVeg [®] peptone No. 3	13.000
Lactose	10.000
Synthetic detergent No. III	2.000
Neutral red	0.020
Sodium citrate	20.000
Ferric ammonium citrate	2.000
Agar	13.500
Final pH (at 25°C)	7.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 70.52 grams in 1000 ml of purified / distilled water. Heat to boiling to dissolve the medium completely. **DO NOT AUTOCLAVE**. Avoid excessive heating as it is detrimental to the medium. Cool to 45-50°C. Mix well and pour into sterile petri plates.

Principle And Interpretation

Deoxycholate Citrate Agar is prepared as per the modified formula of Leifson (1). This medium is used for the isolation and maximum recovery of intestinal pathogens belonging to *Salmonella* and *Shigella* groups from foods (2). However, it is recommended to use less inhibitory medium when *Shigella* have to be isolated (3). The selectivity of this medium permits the use of fairly heavy inocula without danger of overgrowth of *Shigella* and *Salmonella* by other microflora. This medium is similar to deoxycholate agar in comparison but is moderately more selective for enteric pathogens owing to increased concentrations of both citrate and deoxycholate salts. Sodium deoxycholate at pH 7.3 to 7.5 is inhibitory for gram-positive bacteria. Citrate salts, in the concentration included in the formulation, are inhibitory to gram-positive bacteria and most other normal intestinal organisms.

Deoxycholate Citrate Agar, HiVeg[®] is same as Deoxycholate Citrate Agar except that the animal based peptones are completely replaced with vegetable peptones to avoid the BSE/TSE risks associated with animal peptones. For the routine examination of stool and urine specimens, it is suggested that other media such as MacConkey HiVeg[®] Agar (MV082), Bismuth Sulphite HiVeg[®] Agar (MV027) etc. be used in conjunction with this medium. HiVeg[®] infusion is a source of carbon and nitrogen and this ingredient is used because the inhibition of coliforms produced is greater than when an extract or simple peptone is used. HiVeg[®] peptone No. 3 provides carbon, nitrogen, vitamins and minerals. Coliform bacteria and gram-positive bacteria are inhibited or greatly suppressed due to synthetic detergent No. III, sodium citrate and ferric ammonium citrate. Lactose helps in differentiating enteric bacilli, as lactose fermenters produce red colonies while lactose non-fermenters produce colourless colonies. Coliform bacteria, if present form pink colonies on this medium. The degradation of lactose causes acidification of the medium surrounding the relevant colonies and the pH indicator neutral red changes its colour to red. These colonies usually are also surrounded by a turbid zone of precipitated deoxycholic acid due to acidification of the medium. Synthetic detergent No. III combines with neutral red in an acidic environment, causing the dye to go out of the solution (1). The reduction of ferric ammonium citrate to iron sulfide is indicated by the formation of black iron sulfide. *Salmonella* and *Shigella* species do not ferment lactose but *Salmonella* may produce H₂S, forming colourless colonies with or without black centers. Citrate and iron (Fe) combination has a strong hydrolyzing effect on agar when the medium is heated, producing a soft and unelastic agar. If autoclaved the agar becomes soft and almost impossible to streak. *Salmonella* Gallinarum is inhibited if synthetic detergent No. III concentration is increased to 0.1 % or greater (1). Surface colonies of non-lactose fermenters often absorb a little colour (pinkish) from the medium and organisms may be mistaken for coliforms (1).

Type of specimen

Food samples.

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (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 clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Further biochemical identification is required for confirmation of species.
2. Due to nutritional variations some organisms may show poor growth.

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 pinkish beige homogeneous free flowing powder

Gelling

Firm, comparable with 1.35% Agar gel.

Colour and Clarity of prepared medium

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

Reaction

Reaction of 7.05% w/v aqueous solution at 25°C. pH : 7.5±0.2

pH

7.30-7.70

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony	H ₂ S
<i>Enterococcus faecalis</i> ATCC 29212 (00087*)	≥10 ⁴	inhibited	0%		
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	poor	20-30%	pink with bile precipitate	negative reaction
<i>Salmonella</i> Enteritidis ATCC 13076 (00030*)	50-100	good-luxuriant	≥50%	colourless	positive reaction, black centered colonies
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	50-100	good-luxuriant	≥50%	colourless	positive reaction, black centered colonies
<i>Shigella flexneri</i> ATCC 12022 (00126*)	50-100	good	40-50%	colourless	
<i>Escherichia coli</i> ATCC 8739 (00012*)	50-100	poor	20-30%	pink with bile precipitate	negative reaction

<i>Salmonella</i> Abony NCTC 6017 (00029*)	50-100	good-luxuriant	$\geq 50\%$	colourless	positive
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<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	$\geq 10^4$	inhibited	0%		
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Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°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 (4,5).

Reference

1. Leifson, 1935, J. Path. Bact., 40:581.
2. 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.
3. Frieker C.R., 1987, J. Appl. Bact., 63:99.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. 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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