

Technical Information

Deoxycholate Citrate Agar, Modified (Hynes)

Product Code: DM 2074

Application: - Deoxycholate Citrate Agar, Modified (Hynes) is a selective medium recommended for the isolation of *Salmonella* and *Shigella* species.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	5.000
Beef extract	5.000
Lactose	10.000
Sodium citrate	8.500
Ferric citrate	1.000
Sodium deoxycholate	5.000
Sodium thiosulphate	5.400
Neutral red	0.020
Agar	12.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Leifson ⁽¹⁾ developed Deoxycholate Agar as a differential medium containing pure chemicals, citrates and deoxycholate as inhibitors. Leifson's medium has been modified by many authors by several ways. Deoxycholate Citrate Agar, Modified (Hynes) is a differential medium modified by Hynes ⁽²⁾ for the isolation of *Salmonellae* and *Shigellae*. Deoxycholate Citrate Agar, modification consists of more concentrations of inhibitors and media is used in food microbiology ⁽³⁾.

Peptic digest of animal tissue and beef extract provides carbon, nitrogen, vitamins and minerals. Coliform bacteria and gram-positive bacteria are inhibited due to sodium deoxycholate, sodium citrate and ferric 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 causing the pH indicator neutral red to change its colour to red. These colonies usually are also surrounded by a turbid zone of precipitated deoxycholic acid due to acidification of the medium. Sodium deoxycholate combines with neutral red in an acidic environment, causing the dye to go out of the solution with the subsequent precipitation of deoxycholate ⁽¹⁾. The reduction of sodium thiosulphate to sulfide is indicated by the formation of black iron sulfide. *Salmonella* and *Shigella* species do not ferment lactose but *Salmonella* may produce H₂S forming colorless 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 ⁽¹⁾. Surface colonies of non-lactose fermenters often absorb a little colour (pinkish) from the medium and organisms may be mistaken for coliforms ⁽¹⁾.

Methodology

Suspend 51.92 grams of powder media in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. DO NOT AUTOCLAVE OR OVERHEAT. Excessive heating is detrimental.

Quality Control

Physical Appearance

Light yellow to pinkish beige homogeneous free flowing powder

Gelling

Firm, comparable with 1.2% Agar gel.

Colour and Clarity of prepared medium

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

Reaction

Reaction of 5.19% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH range 7.10-7.50

Cultural Response/ characteristics

DM 2074: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU) ₃	Growth	Recovery	Colour of Colony	H2S
Bacillus cereus ATCC 10876	>=10	luxuriant	0%		
Escherichia coli ATCC 25922	50-100	poor- fair	20-30%	red	Negative reaction
Salmonella Enteritidis ATCC 13076	50-100	good- luxuriant	>=50%	colourless	positive reaction, black centered colonies
Salmonella Typhimurium ATCC 14028	50-100	good- luxuriant	>=50%	colourless	positive reaction, black centered colonies
Shigella flexneri ATCC 12022	50-100	good- luxuriant	>=50%	colourless	Negative reaction
Klebsiella pneumoniae ATCC 13883	50-100	poor- fair	20-30%	red	Negative reaction
Shigella sonnei ATCC 25931	50-100	good- luxuriant	>=50%	pink with bile precipitate	Negative reaction
Staphylococcus aureus ATCC 25923	>=10 ³	luxuriant	0%		

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.

Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

1. Leifson, 1935, J. Pathol. Bacteriol., 40:581.
2. Hynes M., 1942, J. Path. Bacteriol., 54, 193-207.
3. Speck M. (Ed.), 1984, Compendium of Methods for the Microbiological Examination of Foods, 2nd ed., APHA, Washington, D.C.

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