Deoxycholate Citrate Agar, Modified (Hynes) M1074

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

Directions

Suspend 51.92 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE OR OVERHEAT. Excessive heating is detrimental.

Principle And Interpretation

Deoxycholate Citrate Agar, Modified (Hynes) is a selective medium used for isolation and identification of Salmonellae and Shigallae. Leifson (1) developed Deoxycholate Agar as a differential medium containing pure chemicals, citrates and deoxycholate as inhibitors. Leifsons 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, Modified consist of more concentrations of inhibitors and 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 or greatly suppressed 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).

Quality Control

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

Cultural Response

M1074: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	**Inoculum (CFU)**	**Growth**	**Recovery**	**Colour of Colony**	**H2S**	
Bacillus cereus ATCC 10876	>=10^3	inhibited	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	inhibited	0%			

Reference

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.

Storage and Shelf Life

Store below 30°C and use freshly prepared medium. Use before expiry date on the label.