

Deoxycholate Citrate Agar**M065**

Deoxycholate Citrate Agar is a selective medium recommended for the isolation of enteric pathogens particularly *Salmonella* and *Shigella* species.

Composition**

Ingredients	Gms / Litre
Heart Infusion solids	10.000
Proteose peptone	10.000
Lactose	10.000
Sodium deoxycholate	5.000
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 distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Avoid excessive heating as it is detrimental to the medium.

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 *Shigellae* 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. For the routine examination of stool and urine specimens, it is suggested that other media such as MacConkey Agar (M082), Bismuth Sulphite Agar (M027) etc. be used in conjunction with this medium.

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.

Heart 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. Proteose peptone 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 ammonium citrate. Dipotassium phosphate buffers the medium. 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. 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 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 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). *Salmonella* Gallinarum is inhibited if sodium deoxycholate 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).

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

Cultural Response

M065: 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	≥10 ³	inhibited	0%			
<i>Escherichia coli</i> ATCC 25922	50-100	poor	20-30%	pink with bile precipitate	negative reaction	
<i>Salmonella Enteritidis</i> ATCC 13076	50-100	good-luxuriant	≥50%	colourless	positive reaction, black centered colonies	
<i>Salmonella Typhimurium</i> ATCC 14028	50-100	good-luxuriant	≥50%	colourless	positive reaction, black centered colonies	
<i>Shigella flexneri</i> ATCC 12022	50-100	good	40-50%	colourless	negative reaction	
<i>Escherichia coli</i> ATCC 8739	50-100	poor	20-30%	pink with bile precipitate	negative reaction	
<i>Escherichia coli</i> NCTC 9002	50-100	poor	20-30%	pink with bile precipitate	negative reaction	
<i>Salmonella Abony</i> NCTC 6017	50-100	good-luxuriant	≥50%	colourless	positive reaction, black centered colonies	
<i>Staphylococcus aureus</i> ATCC 25922	≥10 ³	inhibited	0%			

Reference

1., Leifson, 1935, J. Path. Bact., 40:581.

2., Speck M. (Ed.), 1984, Compendium of Methods for the Microbiological Examination of Foods, 2nd ed., APHA, Washington, D.C.

3., Frieker C.R., 1987, J. Appl. Bact., 63:99.

Storage and Shelf Life

Store below 30°C and the prepared medium at 2 - 8°C. Use before expiry date on the label.