

Deoxycholate Agar**M030**

Deoxycholate Agar is used as a differential medium for the direct count of coliforms in dairy products. Also used for the isolation of enteric pathogens from rectal swabs, faeces and other pathological specimens.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	10.000
Lactose	10.000
Sodium deoxycholate	1.000
Sodium chloride	5.000
Dipotassium phosphate	2.000
Ferric citrate	1.000
Sodium citrate	1.000
Neutral red	0.030
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 45.03 grams in 1000 ml distilled water. Heat, to boiling, to dissolve the medium completely. DO NOT AUTOCLAVE. Avoid excessive or prolonged heating during reconstitution..

Principle And Interpretation

Deoxycholate Citrate Agar is prepared as per the modified formula of Leifson (1). This media is used for the isolation and maximum recovery of intestinal pathogens belonging to *Salmonella* and *Shigella* species (2). The selectivity of medium permits the use of fairly heavy inocula without danger of overgrowth of the *Shigella* and *Salmonella* by other micro-flora.

For the routine examination of stool and urine specimens, it is recommended that other media such as MacConkey Agar (M082), Bismuth Sulphite Agar (M027) etc. be used in conjunction with this medium. It can also be used to streak specimen from Selenite Broth cultures. This is particularly recommended for the detection of *Shigella* and *Salmonella* in the examination of rectal swabs and faeces. These organisms produce colourless colonies on this medium.

Peptic digest of animal tissue provides carbon, nitrogen, vitamins and minerals. Coliform bacteria and gram-positive bacteria are inhibited or greatly suppressed due to sodium deoxycholate and sodium citrate. Sodium chloride maintains the osmotic balance of the medium while 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).

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 pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

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

Reaction

Reaction of 4.5% w/v aqueous solution at 25°C. pH : 7.3±0.2

Cultural Response

M030: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony		
<i>Salmonella Typhi</i> ATCC 6539	50-100	luxuriant	≥50%	colourless		
<i>Staphylococcus aureus</i> ATCC 25923	≥10 ³	inhibited	0%			
<i>Enterococcus faecalis</i> ATCC 29212	≥10 ³	inhibited	0%			
<i>Escherichia coli</i> ATCC 25922	50-100	good	40-50%	pink with bile precipitate		
<i>Salmonella Enteritidis</i> ATCC 13076	50-100	good-luxuriant	≥50%	colourless		
<i>Salmonella Typhimurium</i> ATCC 14028	50-100	good-luxuriant	≥50%	colourless		
<i>Shigella flexneri</i> ATCC 12022	50-100	good	40-50%	colourless		

Reference

1. Leifson, 1935, J. Path. Bacteriol., 40:581.
2. Speck M. L., (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 the prepared medium at 2 - 8°C. Use before expiry date on the label.