

## Deoxycholate Citrate Agar (DM577)

#### Intended Use

Deoxycholate Citrate Agar (DM577) is recommended for selective isolation of enteric pathogens particularly *Salmonella* and *Shigella* species.

### Product Summary and Explanation

Deoxycholate Citrate Agar is a modification of Deoxycholate Agar formulated by Leifson.<sup>(1)</sup> This medium is used for the isolation and maximum recovery of intestinal pathogens belonging to *Salmonella* and *Shigella* groups from foods.<sup>(2)</sup> When *Shigellae* have to be isolated, it is recommended to use less inhibitory medium.<sup>(3)</sup> The selectivity of this medium permits the use of fairly heavy inocula without danger of overgrowth of *Shigella* and *Salmonella* by other microflora. It is suggested that for the routine examination of stool and urine specimens, other media such as MacConkey Agar (DM143), Bismuth Sulphite Agar (DM039) etc. be used in conjunction with this medium. Deoxycholate Agar was an improvement over other media because citrates and sodium deoxycholate worked well as inhibitors. Leifson modified the original medium by increasing the concentration of Sodium Citrate and Sodium Deoxycholate for improved recovery of *Salmonella* spp. and *Shigella* spp. Sodium deoxycholate at pH 7.3 to 7.5 is inhibitory for grampositive 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 effectively* isolates intestinal pathogens by inhibiting coliforms and many *Proteus* spp. This medium is used to screen *Salmonella* spp. and *Shigella* specimens.<sup>(4)</sup>

#### Principles of the Procedure

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. Sodium deoxycholate, sodium citrate and ferric ammonium citrate act as inhibitors for Coliform bacteria and gram-positive bacteria. Dipotassium phosphate serves as buffering agent. Lactose is a fermentable carbohydrate, which helps in differentiating enteric bacilli. Lactose fermenters produce red colonies while lactose nonfermenters produce colourless colonies. Coliform bacteria, if present form pink colonies on this medium. Lactose degradation causes acidification of the medium surrounding the relevant colonies and the pH indicator neutral red changes its colour to red. Acidification of the medium also, results in the formation of a turbid zone of precipitated deoxycholic acid around these colonies. Sodium deoxycholate and neutral red combines in anacidic environment, causing the dye to go out of the solution with the subsequent precipitation of deoxycholate. The reduction of ferric ammonium citrate to iron sulfide is indicated by the formation of black iron sulphide indication of H2S production. 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 that produces a soft and unelastic agar when the medium is heated. If autoclaved the agar becomes soft and almost impossible to streak. Salmonella gallinarum is inhibited if sodium deoxycholate concentration is increased to 0.1 % or greater. Surface colonies of non-lactose fermenters often absorb a little colour (pinkish) from the medium and organisms may be mistaken for coliforms.

Formula	1	Liter
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Ingredients	Gms / Liter
Heart infusion solids	10.00
Proteose peptone	10.00





Lactose	10.00		
Sodium Deoxycholate	5.00		
Neutral red	0.02		
Sodium Citrate	20.00		
Ferric Ammonium citrate	2.00		
Agar 13.50			
Final pH: 7.5 ± 0.2 at 25°C			
Formula may be adjusted and/or supplemented as required to meet performance specifications			

#### Precautions

- 1. For Laboratory Use only.
- 2. IRRITANT. Irritating to eyes, respiratory system, and skin.
- 3. DO NOT AUTOCLAVE OR REMELT. This medium is heat sensitive. Avoid excessive or prolonged heating during reconstitution.

### Directions

- 1. Suspend 70.52 grams of the medium in one liter of distilled water.
- 2. Heat to boiling, to dissolve the medium completely.
- 3. DO NOT AUTOCLAVE. Avoid overheating as it is detrimental to the medium.

### Quality Control Specifications

Dehydrated Appearance	Light yellow to pinkish beige, homogeneous free flowing powder			
Prepared Medium	Reddish-orange colored, clear to slightly opalescent gel forms in Petri plates.			
Reaction of 7.05% solution	pH 7.5 <u>+</u> 0.2 at 25°C			
Gel Strength	Firm, comparable with 1.35% Agar gel			

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

6		Results to be achieved			achieved	
Sr. No.	Organisms	Inoculum (CFU)	Growth	Recovery	Color of colony	H₂S
1.	Enterococcus faecalis ATCC 29212	>=10 <sup>3</sup>	inhibited	0%		
2.	Escherichia coli ATCC 25922	50-100	Poor	20-30%	pink with bile precipitate	negative reaction
3.	Salmonella Enteritidis ATCC 13076	50-100	good- luxuriant	>=50%	Colourless	positive reaction, black centered colonies
4.	Salmonella Typhimurium ATCC 14028	50-100	good- luxuriant	>=50%	Colourless	positive reaction, black centered colonies
5.	Shigella flexneri A TCC 12022	50-100	good	<b>≻=</b> 40-50%	colourless	negative reaction





6.	Escherichia coli ATCC 8739	50-100	poor	20-30%	pink with bile precipitate	negative reaction
7.	Escherichia coli NCTC 9002	50-100	poor	20-30%	pink with bile precipitate	negative reaction
8.	Salmonella Abony NCTC 6017	50-100	good- luxuriant	>=50%	Colorless	positive reaction, black centered colonies
9.	Staphylococcus aureus ATCC 25923	>=10 <sup>3</sup>	inhibited	0%		

The organisms listed are the minimum that should be used for quality control testing.

#### Test Procedure

- 1. Inoculate specimen directly onto surface of medium.
- 2. Incubate plates at  $35 \pm 2^{\circ}C$  for 18-24 hours. Plates can be incubated for an additional 24 hours if no lactose fermenters are observed.

#### Results

- 1. Lactose fermenters produce a pink-red colony with or without a bile precipitate.
- 2. Lactose nonfermenters produce transparent, colorless to light tan or colorless colonies with or without black centers.



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*Escherichia coli* ATCC 25922 - pink-red colonies *Salmonella Enteritidis* ATCC 13076 - colorless colonies with black centers.





#### Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

#### Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

#### Limitations of the Procedure

- 1. Coliform strains may be encountered that will grow on this medium, making it difficult to detect pathogens.
- 2. Heavy inocula should be distributed over the entire surface of the medium to prevent complete masking of pathogens by coliform organisms.
- 3. Consult appropriate texts for detailed information and recommended procedures.

#### Packaging

Product Name : Deoxycholate Citrate Agar. Product Code : DM577 Available Pack sizes : 100gm / 500gm

#### References

- 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.
- 4. Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.). Manual of clinical microbiology, 6<sup>th</sup> ed.American Society for Microbiology, Washington, D.C.

#### Further Information

For further information please contact your local MICROMASTER Representative.



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