



Medium 14. Desoxycholate-Citrate Agar

MM065

Medium 14. Desoxycholate-Citrate Agar is recommended for the a selective isolation and identification of *Salmonellae* in accordance with Indian Pharmacopoeia 2007.

Composition**

Ingredients	Gms / Litre
Peptone	10.000
Beef extract	10.000
Lactose monohydrate	10.000
Trisodium citrate	20.000
Ferric citrate	1.000
Sodium desoxycholate	5.000
Neutral red	0.020
Agar	13.500
pH after heating (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 69.02 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml of purified/distilled water. Heat to boiling to dissolve the medium completely. Agitate to prevent charring. DO NOT AUTOCLAVE /OVERHEAT OR REMELT. The medium is cooled to 45-50°C and plates are poured. Dry the agar surface before use.

Principle And Interpretation

Desoxycholate Citrate Agar is prepared as per the modified formula of Leifson (1) and is also recommended by Indian Pharmacopoeia 2007 (2). This medium is used for the isolation and maximum recovery of intestinal pathogens belonging to *Salmonella* and *Shigella* groups from foods and pharmaceutical products (3). However, it is recommended to use less inhibitory medium when Shigellae have to be isolated (4). *Salmonella*, major causative agent of enteric disease especially food borne toxic infection and typhoid was first observed by Eberth in 1880. This medium is routinely used to check the presence of *Salmonella* in food and pharmaceutical products.

Proteus and other Gram positive organisms are inhibited due to higher concentration of both citrate and deoxycholate salts in this medium. Sodium desoxycholate at pH 7.3 to 7.5 is inhibitory for gram-positive bacteria. Sodium thiosulphate also helps in reactivation of sulphur containing compounds and prevents the desiccation of these compounds during storage. It also forms the substrate for enzyme thiosulphate reductase, which breaks it; to form H₂S. H₂S then reacts with Fe ions in the medium and produces black FeS precipitate. This gives the indicative appearance of colonies with black center. Sodium thiosulphates are also inactivators of halogens and can minimize its toxicity in the testing sample, if any during microbial limit tests. Citrate salt, in the concentration included in the formulation, are inhibitory to gram-positive bacteria and most other normal intestinal organisms. Combination of beef extract and peptone supplies nitrogen, mineral, vitamin factors required for enhanced growth. Lactose monohydrate supplies fermentable carbohydrate source in this medium. Neutral red acts as indicators, in presence of which lactose fermenters like coliform bacteria give pink colonies while lactose non-fermenters give colourless colonies.

Salmonella gives either colourless and opaque colonies with or without black center, while *Shigella* gives colourless colonies without black center indicating absence of H₂S production. Precipitation of deoxycholate by acid produced by lactose fermenters may give a zone of precipitation around the colony. This medium provides essential growth factors for growth of several auxotrophic strains of Paratyphi and Typhi. The selectivity of this medium permits the use of fairly heavy inocula without danger of overgrowth of the *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 (MM082), Bismuth Sulphite Agar (MM027) etc. be used in conjunction with this medium.

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 6.90% w/v aqueous solution after heating. pH : 7.3±0.2

pH

7.10-7.50

Cultural Response

Growth Promotion is carried out in accordance with IP. Cultural response was observed after an incubation at 36-38°C for 18-24 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Cultural Response

Organism	Inoculum (CFU)	Observed Lot value (CFU)	Recovery	Colour of Colony	Incubation temperature	Incubation period
Test for specified microorganism						
<i>Salmonella Typhimurium</i> ATCC 14028	50 -100	25 -100	≥50 %	Colourless and opaque with or without black centres	36 -38 °C	18 -24 hrs
<i>Salmonella Abony</i> NCTC 6017	50 -100	25 -100	≥50 %	Colourless and opaque with or without black centres	36 -38 °C	18 -24 hrs
Cultural Response Additional microbiological testing						
<i>Salmonella Enteritidis</i> ATCC 13076	50 -100	25 -100	≥50 %	Colourless and opaque with or without black centres	36 -38 °C	18 -24 hrs
<i>Salmonella Typhi</i> ATCC 6539	50 -100	25 -100	≥50 %	Colourless and opaque with or without black centres	36 -38 °C	18 -24 hrs
<i>Escherichia coli</i> NCTC 9002	50 -100	0 -10	0 -10 %	Pink with bile precipitate	36 -38 °C	18 -24 hrs
<i>Escherichia coli</i> ATCC 8739	50 -100	0 -10	0 -10 %	Pink with bile precipitate	36 -38 °C	18 -24 hrs
<i>Shigella flexneri</i> ATCC 12022	50 -100	0 -10	0 -10 %	colourless	36 -38 °C	18 -24 hrs
<i>Enterococcus faecalis</i> ATCC 29212	≥10 ³	0	0%		36 -38 °C	18 -24 hrs

Storage and Shelf Life

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

Reference

1. Leifson, 1935, J. Path. Bact., 40:581.
2. Indian Pharmacopoeia, 2007, Govt. of India, Ministry of Health and Family Welfare, New Delhi, India.
3. Speck M. (Eds.), 1984 Compendium of Methods for the Microbiological Examination of Foods, 2nd ed., APHA, Washington, D.C.
4. Frieker C.R., 1987, J. Appl. Bact., 63:99.

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