Christensen Citrate Agar

M143

Christensen Citrate Agar is used for the differentiation of enteric pathogens and coliforms on the basis of citrate utilization.

Composition**

Ingredients	Gms / Litre
Yeast extract	0.500
L-Cysteine hydrochloride	0.100
Sodium citrate	3.000
Dextrose	0.200
Monopotassium phosphate	1.000
Sodium chloride	5.000
Phenol red	0.012
Agar	15.000
Final pH (at 25°C)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 24.8 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense into test tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool the tubes in a slanted position.

Principle And Interpretation

Christensen Citrate Agar is a modification of Christensen Iron Agar (1), which has the same formulation except the additional sodium thiosulphate and ferric ammonium citrate as described by Edwards and Ewing (2). Christensen reported that all members of genera *Escherichia, Enterobacter, Citrobacter* and *Salmonella* were capable of utilizing citrate as a source of energy while *Shigella* species failed to utilize citrate. Edward and Ewing (2) recommended the use of Triple Sugar Iron Agar (M021) for the determination of hydrogen sulphide production and Christensen Citrate Agar for citrate utilization.

Organisms that metabolize citrate as a sole source of carbon cleave citrate to oxaloacetate and acetate via the citritase enzyme. Another enzyme, oxaloacetate decarboxylase, then converts oxaloacetate to pyruvate and CO2. Further, this CO2 combines with sodium and water to form sodium carbonate, an alkaline compound (3). As a result, the pH of medium rises and the indicator, phenol red changes from orange red to cerise. Presence of the cerise colour indicates a positive finding for citrate utilization.

Medium constituent yeast extract provide the necessary nutrients mainly nitrogenous and vitamins for the growth of the organisms. L-cysteine hydrochloride is a reducing agent. Dextrose is the fermentable carbohydrate. Sodium citrate is the energy source for citrate utilizing organisms. Care should be taken while inoculating, as, a too heavy inoculum may give a false positive result (4).

Quality Control

Appearance
Light yellow to light pink homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Orange red coloured, very slightly opalescent gel forms in tubes as slants

Reaction
Reaction of 2.48% w/v aqueous solution at 25°C. pH : 6.9±0.2

pH
6.70-7.10

Cultural Response

M143: Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Citrate utilization (Colour of slant)
Enterobacter aerogenes ATCC 13048	50-100	luxuriant	positive reaction, cerise colour
Escherichia coli ATCC 25922	50-100	luxuriant	negative reaction, no colour change
Klebsiella pneumoniae ATCC 13883	50-100	luxuriant	weakly positive, orange-pink colour
Salmonella Typhimurium ATCC 14028	50-100	luxuriant	positive reaction, cerise colour
Salmonella Enteritidis ATCC 13076	50-100	luxuriant	positive reaction, cerise colour
Shigella flexneri ATCC 12022	50-100	luxuriant	negative reaction, no colour change
Shigella sonnei ATCC 25931	50-100	luxuriant	negative reaction, no colour change

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.Christensen W.B., 1949, Research Bull., Weld County Health Dept., Greenley Co., 1:3.
2.Edwards P.R. and Ewing W. H., 1955 and 1962, Identification of Enterobacteriaceae Minneapolis, Burgess Publishing Co., pg. 179 and 242.
3.Horward B., 1994, Clinical and Pathogenic Microbiology, 2nd ed., Mosby Year Book, Inc.
4.Branson D., 1972, Methods in Clinical Bacteriology, Springfield, III: C. Thomas, 15.

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