Christensen Citrate Sulphite Agar

M495

Christensen Citrate Sulphite Agar is used for the differentiation of enteric bacilli on the basis of citrate utilization and hydrogen sulphide production.

Composition**

Ingredients	Gms / Litre
Sodium citrate	3.000
Dextrose	0.200
Yeast extract	0.500
L-Cysteine hydrochloride	0.100
Ferric ammonium citrate	0.400
Potassium phosphate	1.000
Sodium chloride	5.000
Sodium thiosulphate	0.080
Phenol red	0.012
Agar	14.000
Final pH (at 25°C)	6.7±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 24.29 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense into test tubes. Sterilize by autoclaving at 12 to15 lbs pressure (118 to 121°C) for 15 minutes. Cool the tubes in slanted position to give slants with generous butts.

Principle And Interpretation

Christensen Citrate Sulphite Agar is a modification of the Christensen Iron Agar (1). This modification was described by Edwards and Ewing (2). Christensen reported that all members of genera *Escherichia, Enterobacter, Citrobacter* and *Salmonella* as well as Alkalescens-Dispar were capable of utilizing citrate as a source of energy while *Shigella* species failed to utilize citrate.

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).

The reduction of ferric ammonium citrate to iron sulphide by H2S producing organisms is indicated by blackening of the medium. Sodium thiosulphate enhances H2S production. Strong positive cultures upon prolonged incubation turn the entire butt black. Some members of *Salmonella* like *Salmonella* Typhi are weakly positive and require 2-5 days for hydrogen sulphite production.

Quality Control

Appearance

Light yellow to light pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.4% Agar gel.

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

Reaction
Reaction of 2.42% w/v aqueous solution at 25°C. pH : 6.7±0.2

pH
6.50-6.90

Cultural Response
M495: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Citrate Utilisation	H2S
Enterobacter aerogenes ATCC 13048	50-100	luxuriant	positive reaction, cerise colour	negative reaction,no colour change
Escherichia coli ATCC 25922	50-100	luxuriant	negative reaction, no colour change	negative reaction,no colour change
Salmonella Typhimurium ATCC 14028	50-100	luxuriant	positive reaction, cerise colour	positive reaction, blackening of medium
Salmonella Enteritidis ATCC 13076	50-100	luxuriant	positive reaction, cerise colour	positive reaction, blackening of medium
Klebsiella pneumoniae ATCC 13883	50-100	luxuriant	weakly positive, orange-pink colour	negative reaction,no colour change
Shigella flexneri ATCC 12022	50-100	luxuriant	negative reaction, no colour change	negative reaction,no change
Shigella sonnei ATCC 25931	50-100	luxuriant	negative reaction, no colour change	negative reaction,no change

Storage and Shelf Life
Store below 30°C in tightly cloased 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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