Christensen Citrate Sulphite agar, w/ 1.5% agar

M495F

Christensen Citrate Sulphite Agar, w/1.5% agar is used for the differentiation of enteric bacilli on the basis of citrate utilization and hydrogen sulphide production in accordance with FDA BAM, 1998.

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	15.000
Final pH (at 25°C)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 25.29 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 in a slanted position to give slants with generous butts.

Principle And Interpretation

Christensen Citrate Sulphite Agar, w/1.5% agar is used for the differentiation of enteric bacilli on the basis of citrate utilization and hydrogen sulphide production in accordance with FDA BAM, 1998(1). Christensen Citrate Sulphite Agar was formulated by Edwards and Ewing (2,3) as a modification of the Christensen Iron Agar (4). 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 (5). 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.

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 (6). 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.

According to FDA BAM, recovery of *Shigella* is done in two different ways. First is the conventional method wherein the organism is grown in a selective media such as Shigella Broth Base (M1326) with novobiocin, isolated in selective media such as MacConkey Agar (M081D) and further confirmed using biochemical tests. In the second method, *Shigella* is identified using DNA hybridization technology. In the conventional method, the organisms isolated in selective agar are confirmed using various biochemical reactions including citrate utilization test. For citrate utilization test, inoculate the isolated colonies into Christensen Citrate Sulphite Agar, w/1.5% agar (M495F). *Shigella* does not utilize citrate and give negative citrate utilization reaction.

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 2.53% w/v aqueous solution at 25°C. pH : 6.9±0.2

pH
6.70-7.10

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

Cultural Response

Organism	Inoculum (CFU)	Growth	Citrate Utilisation	H2S
Cultural Response				
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 boydii ATCC 12030	50-100	luxuriant	negative reaction, no colour change	negative reaction,no change
Shigella dysenteriae ATCC 13313	50-100	luxuriant	negative reaction, no colour change	negative reaction,no 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.FDA, U.S. 1998. Bacteriological Analytical Manual. 8 ed. Gaithersburg, MD: AOAC International.
2.Edward, P.R. and Fife M.A. 1961. Appl. Microbiol.
3.Edwards P.R. and Ewing W.H., 1955, Minneapolis, Burgess Publishing Co.
4.Christensen W.B., 1949, Research Bull., Weld County Health Dept., Greenley Co., 1:3.
5.Howard, B. J. 1994. Clinical and Pathogenic Microbiology. 2 ed.: Mosby Year Book.
6.Branson. 1972. Charles C. Thomas, (ed.): Springfield.

Revision : 1 / 2015