

Technical Information

Christensen Citrate Sulphite Agar

Product Code: DM 1495

Application: - Christensen Citrate Sulphite Agar is recommended 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

Principle & Interpretation

Christensen Citrate Sulphite Agar is a modified of type of the Christensen Iron Agar (1). This modification was formulated 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 in the medium. Dextrose act as 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.

Methodology

Suspend 24.29 grams of dehydrated powder media in 1000 ml distilled water. Mix thoroughly & heat to boil to dissolve the medium completely. Dispense into test tubes. Sterilize by autoclaving at 12 to 15 lbs pressure (118 to 121°C) for 15 minutes. Cool the tubes in slanted position to give slants with generous butts.

Quality Control

Appearance

Light yellow to light pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.4% Agar gel.

Colour and Clarity

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 Range

6.50-6.90

Cultural Response

DM 1495: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Citrate Utilisation	H ₂ S
<i>Enterobacter aerogenes</i> ATCC 13048	50-100	luxuriant	positive reaction, cerise colour	negative reaction, no colour change
<i>Escherichia coli</i> ATCC 25922	50-100	luxuriant	negative reaction, no colour change	negative reaction, no colour change
<i>Salmonella Typhimurium</i> ATCC 14028	50-100	luxuriant	positive reaction, cerise colour	positive reaction, blackening of Medium
<i>Salmonella Enteritidis</i> ATCC 13076	50-100	luxuriant	positive reaction, cerise colour	positive reaction, blackening of Medium
<i>Klebsiella pneumoniae</i> ATCC 13883	50-100	luxuriant	weakly positive, orange-pink Colour	negative reaction, no colour change
<i>Shigella flexneri</i> ATCC 12022	50-100	luxuriant	negative reaction, no colour change	negative reaction, no colour change
<i>Shigella sonnei</i> ATCC 25931	50-100	luxuriant	negative reaction, no colour change	negative reaction, no colour change

Storage and Shelf Life

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

Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

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, Ill: C. Thomas, 15.

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
- The product conform solely to the technical information provided in this booklet and to the best of knowledge research and development work carried at CDH is true and accurate
- Central Drug House Pvt. Ltd. reserves the right to make changes to specifications and information related to the products at any time.
- Products are not intended for human or animal diagnostic or therapeutic use but for laboratory, research or further manufacturing of diagnostic reagents extra.
- Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents. Do not use the products if it fails to meet specifications for identity and performance parameters.