TM 066 – CHRISTENSEN CITRATE SULPHITE AGAR

INTENDED USE

For differentiation of enteric bacilli based on citrate utilization and H_2S production.

PRODUCT SUMMARY AND EXPLANATION

Christensen Citrate Sulphite Agar is a modification of the Christensen Iron Agar. This modification was described by Edwards and Ewing. 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 CO_2. Further, this CO_2combines with sodium and water to form sodium carbonate, an alkaline compound. 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.

Care should be taken while inoculating, as, a too heavy inoculum may give a false positive result. The reduction of ferric ammonium citrate to iron sulphide by H_2S producing organisms is indicated by blackening of the medium. Sodium thiosulphate enhances H_2S 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.

COMPOSITION

Ingredients	Gms / Ltr
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

PRINCIPLE

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.

INSTRUCTION FOR USE

- Dissolve 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 psi pressure (118 to 121°C) for 15 minutes.
- Cool the tubes in slanted position to give slants with generous butts.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Light yellow to light pink homogeneous free flowing powder.
Appearance of prepared medium : Orange red coloured, very slightly opalescent gel forms in tubes as slants.
pH (at 25°C) : 6.7±0.2

INTERPRETATION

Cultural characteristics observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Citrate Utilisation	H_2S	Incubation Temperature	Incubation Period
Enterobacter aerogenes	13048	50-100	Luxuriant	>=70%	Positive reaction, cerise colour	Negative reaction, no colour change	35-37°C	18-24 Hours
Escherichia coli	25922	50-100	Luxuriant	>=70%	Negative reaction, no colour change	Negative reaction, no colour change	35-37°C	18-24 Hours
Salmonella Typhimurium	14028	50-100	Luxuriant	>=70%	Positive reaction, cerise colour	Positive reaction, blackening of medium	35-37°C	18-24 Hours
Salmonella Enteritidis	13076	50-100	Luxuriant	>=70%	Positive reaction, cerise colour	Positive reaction, blackening of medium	35-37°C	18-24 Hours
Klebsiella pneumoniae	13883	50-100	Luxuriant	>=70%	Weakly positive, orange-pink colour	Negative reaction, no colour change	35-37°C	18-24 Hours
Shigella flexneri	12022	50-100	Luxuriant	>=70%	Negative reaction, no colour change	Negative reaction, no colour change	35-37°C	18-24 Hours
Shigella sonnei	25931	50-100	Luxuriant	>=70%	Negative reaction, no colour change	Negative reaction, no colour change	35-37°C	18-24 Hours

PACKAGING:

In pack size of 500 gm bottles.

STORAGE

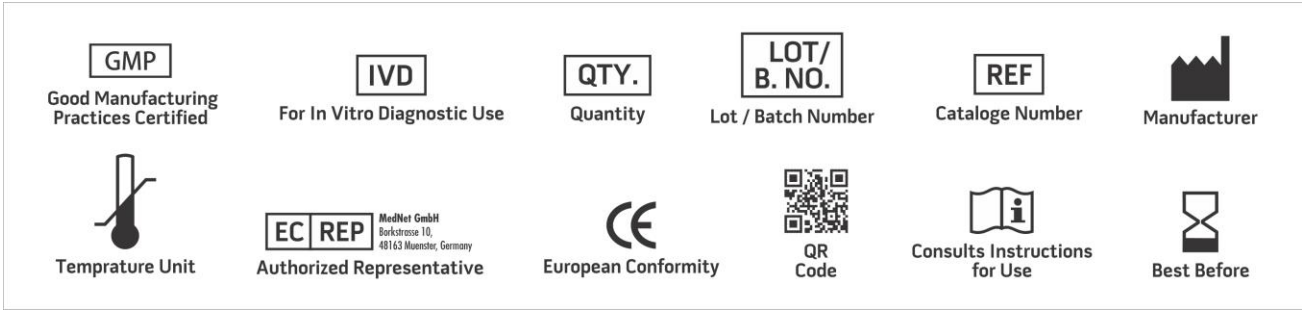
Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

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.



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

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