TM 2039 – CHRISTENSEN CITRATE SULPHITE AGAR, W/ 1.5% AGAR

INTENDED USE

For differentiation of enteric bacilli on the basis of citrate utilization and hydrogen sulphide production in accordance with FDA BAM, 1998.

PRODUCT SUMMARY AND EXPLANATION

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. Christensen Citrate Sulphite Agar was formulated by Edwards and Ewing (2,3) as a modification of the Christensen Iron Agar. 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_2 combines 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.

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 with novobiocin, isolated in selective media such as MacConkey Agar 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. *Shigella* does not utilize citrate and give negative citrate utilization reaction.

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	15.000

PRINCIPLE

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 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 psi pressure (121°C) for 15 minutes.
- Cool in a 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) : 6.9±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 boydii	12030	50-100	Luxuriant	>=70%	Negative reaction, no colour change	Negative reaction, no colour change	35-37°C	18-24 Hours
Shigella dysenteriae	13313	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.

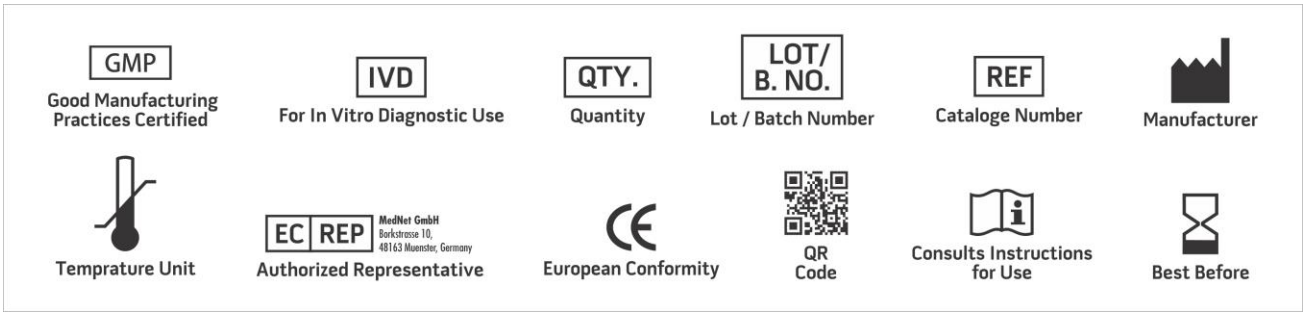
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

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

REFERENCES

1.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.



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

***For Lab Use Only**

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