Technical Information

Simmons Citrate Agar

Product Code: DM 1099

Application: Simmons Citrate Agar is recommended for differentiation the members of *Enterobacteriaceae* on the basis of citrate utilization.

Composition**

Ingredients	Gms / Litre
Magnesium sulphate	0.200
Ammonium dihydrogen phosphate	1.000
Dipotassium phosphate	1.000
Sodium citrate	2.000
Sodium chloride	5.000
Bromothymol blue	0.080
Agar	15.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

On the basis of citrate utilization as sole carbon source these media are used for the differentiation between *Enterobacteriaceae* and the members of aerogenes group. Initially the citrate medium was developed by Koser [1] containing ammonium salt as the only nitrogen source and citrate as the only carbon source for differentiating *Escherichia coli* and *Enterobacter aerogenes* by IMViC tests. Later Simmons [2] modified Kosers formulation by adding agar and bromo thymol blue [3] to the original media It is aso recommended by APHA [4] for differentiation of member of *Enterobacteriaceae*.

Ammonium dihydrogen phosphate and sodium citrate serve as the sole nitrogen and carbon source respectively. Microorganisms also use inorganic ammonium salts as their sole nitrogen source. Metabolism of these salts causes the medium to become alkaline, indicated by a change in colour of the pH indicator from green to blue. Bromothymol blue is the pH indicator. The medium should be freshly prepared because in dry conditions, changes in colour may appear even before inoculation, especially at the bottom of the slant.

Methodology

Suspend 24.28 grams of powder media in 1000 ml distilled water. Shake well & heat, to dissolve the medium completely. Mix well and distribute in tubes or flasks. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Quality Control

Physical Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Forest green coloured slightly opalescent gel forms in tubes as slants

Reaction

Reaction of 2.43% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH Range:- 6.60-7.00

Cultural Response/Characteristics

DM1099: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum(CFU)	Growth	Citrate utilisation
Enterobacter aerogenes ATCC 13048	50-100	good-luxuriant	positive reaction, blue colour
Escherichia coli ATCC 25922	>=10^3	Inhibited	-
Salmonella Choleraesuis ATCC 12011	50-100	good-luxuriant	positive reaction, blue colour
Salmonella Enteritidis ATCC 13076	50-100	luxuriant	positive reaction, blue colour
Salmonella Typhi ATCC 6539	50-100	fair-good	negative reaction, green colour
Salmonella Typhimurium ATCC 14028	50-100	good-luxuriant	positive reaction, blue colour
Shigella dysenteriae ATCC 13313	>=10^3	Inhibited	-

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

1. Koser, 1923, J. Bact., 8:493.
2. Simmons, 1926, J. Infect. Dis., 39:209.
3. MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
4. Eaton A. D., Clesceri L. S., Rice E. W., and Greenberg A W., (Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st Ed., APHA, Washington, D.C.

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