Simmons Citrate Agar ISO

Cat. 1014

For differentiation of Enterobacteriaceae on the basis of citrate utilization

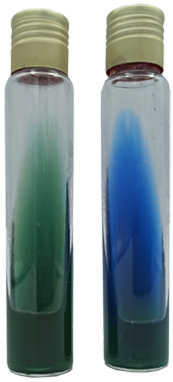
Practical information

Aplications	Categories
Confirmation	Enterobacteria

Industry: Clinical / Food

Regulations: ISO 10273

CE IVD



Principles and uses

Simmons Citrate Agar is used to differentiate Gram negative enteric bacilli on the basis of Sodium citrate as a source of carbon and inorganic Ammonium salt as a source of nitrogen. It is recommended for the differentiation of coliforms isolated from water and clinical samples.

It is used in the same manner as Koser Citrate Broth (Cat. 1200) for the utilization of citrate as one of the IMVIC reactions. Magnesium sulfate is a cofactor for various metabolic reactions. Sodium chloride maintains the osmotic balance. Dipotassium phosphate acts as a buffer system. Bromothymol blue is a pH indicator. Ammonium dihydrogen phosphate is the sole source of nitrogen. Sodium citrate is the sole source of carbon. Bacteriological agar is the solidifying agent.

Only those organisms capable of utilizing citrate as a source of carbon grow and produce a color change from green to blue (alkaline), whilst when no citrate utilization takes place (negative test), the color of the medium remains the same.

Escherichia coli, alongside Shigella, Yersinia and Edwardsiella species, do not grow on the medium. Serratia and most Enterobacter, Citrobacter, Klebsiella, Proteus and Providencia species, except for Morganella morganii and Klebsiella rhinoscleromatis, utilize citrate and produce the typical blue coloration.

Simmons Citrate Agar is also used to differentiate citrate-positive Salmonella enteritidis and members of Salmonella subgenus II, III and IV from the citrate-negative Salmonella typhi, Salmonella paratyphi A, Salmonella pullorum and Salmonella gallinarum.

ISO 10273 recommends this medium for the confirmation of Yersinia enterocolitica. The medium remains green since Yersinia enterocolitica does not use citrate as the sole source of carbon.

Formula in g/L

Bromthymol blue	0,08	Bacteriological agar	15
Dipotassium phosphate	1	Magnesium sulfate	0,2
Sodium chloride	5	Sodium citrate	2
Ammonium Dihydrogen Phosphate	1		

Typical formula g/L * Adjusted and/or supplemented as required to meet performance criteria.

Preparation

Suspend 24,3 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes and sterilize in autoclave at 121 ºC for 15 minutes. Allow cooling in a slanted position in order to obtain short butts of 1-1,5 cm. depth. Alternatively, the medium can be poured into Petri dishes.

Instructions for use

» For clinical diagnosis, the type of sample is bacteria isolated from feces.

- Inoculate the slant agars with the growth of a pure culture using a diluted inoculum.
- Incubate all tubes at 35±2 °C for 24-48 hours in an aerobic atmosphere.
- Reading and interpretation of results

» For other uses not covered by the CE marking:

Confirmation of pathogenic Yersinia enterocolitica according to ISO 10273:
- Obtain a pure colony of the test organism.
- Streak with the aid of a loop the surface of the Simmons Citrate Agar plate or, streak the slanted surface and stab the base of the Simmons Citrate Agar tube.
- Incubate at 35±2 ºC for 24-48 hours.
- For cultivation of Yersinia enterocolitica incubate at 30 ºC for 24±2 hours.
- If good results are not obtained, as in the case of some Providencia strains, incubate for 7 days.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Green	Bluish-green	6,9 ± 0,2

Microbiological test

Incubation conditions: (35±2 ºC / 24-48 h) / Yersinia enterocolitica (30 ºC / 24±2 h)

Microorganisms	Specification	Characteristic reaction
Klebsiella aerogenes ATCC 13048	Good growth	Positive reaction, blue color of the medium
Salmonella enteritidis ATCC 13076	Good growth	Positive reaction, blue color of the medium
Shigella dysenteriae ATCC 13313	Inhibition	Negative reaction, green color of the medium
Salmonella typhimurium ATCC 14028	Good growth	Positive reaction, blue color of the medium
Salmonella typhi ATCC 19430	Inhibition	Negative reaction, green color of the medium
Escherichia coli ATCC 25922	Inhibition	Negative reaction, green color of the medium
Yersinia enterocolitica ATCC 27729	Inhibition	Negative reaction, green color of the medium.

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

Simmons. J. Inf. Dis. 39:209, 1926. Standard Methods for the Examination of Water and Wastewater. Eleventh Edition. APHA Inc. New York, 1960. Edwards & Ewing. Enterobacteriaceae. USPHS. Publications 743. Washington, 1972.
Torregrosa and Ortiz, Pediatrics 59:35. 1961.
ISO 10273. Microbiology of food and animal feeding stuffs — Horizontal method for the detection of presumptive pathogenic Yersinia enterocolitica