SIMMONS CITRATE AGAR (ISO 10273:2003) TM 348

INTENDED USE
For differentiation of Enterobacteriaceae on the basis of citrate utilization.

COMPOSITION

Ingredients	Gm\Ltr.
Agar	15.000
Sodium chloride	5.000
Sodium citrate	2.000
Dipotassium phosphate	1.000
Ammonium dihydrogen phosphate	1.000
Magnesium sulphate	0.200
Bromothymol blue	0.080

PRODUCT SUMMARY AND EXPLANATION

SIMMONS CITRATE AGAR is used for the differentiation between Enterobacteriaceae and the members of *aerogenes* group on the basis of citrate utilization as sole carbon source. It is recommended for the differentiation of coliforms isolated from water and clinical samples.
The medium is virtually a solidified form of Koser citrate medium which, in its original form, suffered from the disadvantage that false appearance of growth occurred when large inocula were employed. The addition of bromothymol blue indicator to the medium was a distinct improvement.
Simmons Citrate Agar complies with the recommendations of the APHA.
ISO 10273 recommends this medium for the confirmation of *Yersinia enterocolitica*. Inoculate and incubate at 30˚C during 24 hours. The medium remains green since *Yersinia enterocolitica* does not use citrate as the sole source of carbon.
Simmons Citrate Agar is recommended for differentiation of enteric Gram-negative bacilli from laboratory specimens, water samples, and food samples.

PRINCIPLE

Simmons Citrate agar is used to test an organism's ability to utilize citrate as a source of energy. 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. Dipotassium Phosphate act as a buffer. Sodium chloride maintains the osmotic balance of the medium. Magnesium Sulfate is a cofactor for a variety of metabolic reactions.

INSTRUCTION FOR USE

1. Dissolve 24.28 grams in 1000 ml distilled water.
2. Gently heat with constant stirring to dissolve the medium completely.
3. Mix well and distribute in tubes or flasks.
4. Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes.
5. After autoclaving, allow medium to solidify in a slanted position.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder: Cream to yellow colour, homogeneous free flowing powder.

Appearance of prepared medium: Forest green colour, slightly opalescent gel.

pH (at 25°C): 6.8 ± 0.2.

INTERPRETATION:

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

Microorganisms	ATCC	Inoculum (CFU/ml)	Growth	Citrate utilization
Enterobacter aerogenes	13048	50 - 100	Good-Luxuriant	Positive reaction, Blue colour
Salmonella enteritidis	13076	50 - 100	Good-Luxuriant	Positive reaction, Blue colour
Salmonella typhimurium	14028	50 - 100	Good- Luxuriant	Positive reaction, Blue colour
Salmonella typhi	6539	50 - 100	Fair - Good	Negative reaction, Green colour
Shigella dysenteriae	13313	≥ 10^3	Inhibited	------
Escherichia coli	25922	≥ 10^3	Inhibited	------
Yersinia enterocolitica	27729	≥ 10^3	Inhibited	------

STORAGE & STABILITY

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers below 25°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.

REFERENCES

1. Koser, S. A. 1923. Utilization of the salts of organic acids by the colon-aerogenes group. J. Bacteriol. 8:493.
2. Simmons, J. S. 1926. A culture medium for differentiating organisms of typhoid-colon aerogenes groups and for isolation of certain fungi. 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. Revision : 03 / 2017
5. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
6. ISO 10273. Microbiology of food and animal feeding stuffs — Horizontal method for the detection of presumptive pathogenic Yersinia enterocolitica

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