

Simmons Citrate Agar

Solid medium used for the differentiation between members of *Enterobacteriaceae* family based on the utilization of citrate as the only source of carbon.

REF: BS.1/SC01.100.0100	100 Gram	REF: BS.1/SC01.250.0250	250 Gram
REF: BS.1/SC01.500.0500	500 Gram		

CLINICAL SIGNIFICANCE

Citrate utilization test is commonly employed as part of a group of tests, the IMViC (Indole, Methyl Red, VP and Citrate) tests that distinguish between members of the *Enterobacteriaceae* family based on their metabolic by-products. Citrate utilization can be used to differentiate between coliforms such as *Klebsiella aerogenes* (Positive Citrate utilization) which occur naturally in the soil and in aquatic environments from fecal coliforms such as *Escherchia coli* (Negative Citrate utilization) whose presence would indicate fecal contamination.

METHOD PRINCIPLE

Ammonium Dihydrogen Phosphate is the sole source of nitrogen. Dipotassium Phosphate acts as a buffer. Sodium Chloride maintains the osmotic balance of the medium. Sodium Citrate is the sole source of carbon in this medium. Magnesium Sulfate is a cofactor for a variety of metabolic reactions. Agar is the solidifying agent. Organisms capable of utilizing ammonium dihydrogen phosphate and citrate will grow unrestricted on this medium. If citrate can be used, the microbe will accumulate alkaline/basic byproducts. When the bacteria metabolize citrate, the ammonium salts are broken down to ammonia, which increases alkalinity. The shift in pH turns the bromthymol blue indicator in the medium from green to blue above pH 7.6.

MEDIA COMPOSITION

Item	Formula per liter of medium
Magnesium sulphate	0.2 gm
Ammonium dihydorgen phosphate	1 gm
Dipotassium phosphate	1 gm
Sodium citrate	2 gm
Sodium chloride	5 gm
Bromothymol blue	0.08 gm
Agar	15 gm

PRECAUTIONS AND WARNINGS

Media to be handled by entitled and professionally educated person. Do not ingest or inhale.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment.

For further information, refer to the Simmons Citrate Agar material safety data sheet.

STORAGE AND STABILITY

BioScien Simmons Citrate Agar should be stored between 10-30°C in a firmly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to avoid lump development due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in a dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Final pH 6.8 ± 0.2 at 25°C

PREPARATION

Suspend 24.28 grams in 1 liter distilled water. Heat, to boiling, to dissolve the medium completely. Mix well and distribute in appropriate containers. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Deterioration

The color of **BioScien** Simmons Citrate Agar is cream to yellow homogeneous free flowing powder. Prepared Media is forest green in color. If there are any physical changes for powder or signs of deterioration (shrinking, cracking, or discoloration), and contaminations for hydrated media, discard the medium.

SPECIMEN

Isolated microorganisms from clinical and non-clinical samples (food and water samples)

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile petri dishes/Test tubes
- Incubator
- Autoclave

PERFORMANCE CHARACTERISTICS

Cultural characteristics observed after incubation at 35-37°C for 18-24H.

Microorganism	Result	Citrate Utilization
<i>Citrobacter freundii</i> ATCC 43864	Luxuriant growth	Positive and gives blue color
<i>Klebsiella (Enterobacter) aerogenes</i> (ATCC 13048)	Luxuriant growth	Positive and gives blue color
<i>Salmonella Typhi</i> ATCC 6539	Fair-good growth	Negative and gives green color
<i>Salmonella Typhimurium</i> ATCC 14028	Luxuriant growth	Positive and gives blue color
<i>Shigella dysenteriae</i> ATCC 13313	Inhibited	-
<i>Escherichia coli</i> ATCC 25922	Inhibited	-
<i>Salmonella Enteritidis</i> ATCC 13076	Luxuriant growth	Positive and gives blue color

QUALITY CONTROL

To ensure adequate quality control, it is recommended that positive and negative control included in each run. If control still out of range please contact **BioScien** technical support.

REFERENCES

1. Simmons, 1926, J. Infect. Dis., 39:209.
2. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C
3. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
4. MacFaddin, J. D. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.
5. www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalmanualBAM/default.htm.
6. Vanderzant, C., and D. F. Splittstoesser (eds.). 2015. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
7. Isenberg, H. D. (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.

SYMBOLS IN PRODUCT LABELLING	
IVD	For in-vitro diagnostic use
LOT	Batch Code/Lot number
REF	Catalogue Number
	Temperature Limitation
	Expiration Date
	Manufactured by
	Number of <n> test in the pack
	Caution
	Do not use if package is damaged
	Consult Instruction for use



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