

TECHNICAL DATA SHEET

Simmons Citrate Agar

Principle

Simmons Citrate Agar is a modification of Koser's medium (1923). Simmons citrate agar is composed of magnesium sulphate, ammonium dihydrogen phosphate, dipotassium phosphate, sodium citrate, sodium chloride, bromothymol blue and agar. Magnesium sulfate is a cofactor for variety of metabolic reactions for growth and reproduction of microorganisms. Ammonium dihydrogen phosphate is the source of nitrogen. Dipotassium Phosphate acts as a buffer. Sodium Citrate is source of carbon. Sodium Chloride maintains the osmotic balance. Bromothymol blue act as pH indicator. Agar is solidifying agent. The microorganisms have ability to utilize citrate as a source of energy and capable of utilizing ammonium dihydrogen phosphate will grow on this medium. The citrate metabolizing microbes accumulate alkaline by products by synthesizing citrate permease, which convert citrate to pyruvate. The synthesized pyruvate is used by the organisms in metabolic cycle for energy production. The citrate metabolizing bacteria, break down the ammonium salts into the ammonia, result rise in alkalinity of the medium. The shift in pH turns the bromothymol blue indicator in the medium from green to blue above pH 7.6. Simmons citrate agar is recommended by APHA and BIS for the differentiation between *Enterobacteriaceae*.

Use: For the differentiation of Enterobacteriaceae based on citrate utilization.

Contents*

Ingredients	Gram/Liter
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
pH at 25°C	6.8 ± 0.1

* Formula adjusted for optimum performance and parameters

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Directions: Dissolve 24.28 grams in 1000 ml distilled water. Boil to dissolve the medium completely and sterilize by autoclaving at 15 lbs. pressure (121°C) for 15 min, cool it to 42-45 °C and distribute aseptically in test tubes. Slant the tubes to have a small butt and slant, allow to solidify. Ensure complete solidification and inoculate test sample aseptically.

Specimens types analyzed

Pharmaceutical samples, clinical and non-clinical samples etc.

Precautions to be taken

These microbial media are intended for the in-vitro use only. All the handling, experiments, storage, and discarding should be performed with the help of skilled and knowledgeable technicians and as per the established guidelines. The material should be disposed only after proper sterilization by autoclaving. Please go through the MSDS of the media to avoid any accidents or in emergency.

Performance and Evaluation

The expected performance of the medium is liable to use as per the direction on the label when stored at optimum conditions and within expiry date.

Quality Control

Appearance	Light yellow colored free flowing, homogeneous powder
Reaction of 2.42% solution	6.8 ±0.2 at 25°C
pH	6.70 - 6.90
Gelling	Firm comparable with 1.5% agar gel
Color and clarity of ready medium	Forest green colored, slightly opalescent gel
Growth Promotion properties	Best at ≤ 100 CFU at 32-37 °C for 18-72 h
Indicative properties	Optimum at ≤ 100 CFU at 32-37 °C for 18-48 h
Negative control	Performed using sterile distilled water

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Different Microbial Response: Cultural characteristics observed after incubation at 35±2°C for 18-24 hours. Inoculum 50-100 CFU.

Organism	ATCC	Growth	Color of medium around the colonies	Citrate utilization
<i>Escherichia coli</i>	8739	Poor	No color change due to growth inhibition	Negative
<i>Salmonella typhimurium</i>	14028	Good	Blue color formed	Positive
<i>Salmonella enteritidis</i>	13076	Good	Blue color formed	Positive
<i>Klebsiella aerogenes</i>	13048	Good	Blue color formed	Positive

Storage and Shelf Life: The product is highly hygroscopic; keep the container tightly closed at all times and store it properly as per the conditions mentioned on the label. The declared expiry is valid only when stored as per the conditions mentioned on the label.

Note: Sterilize media immediately after reconstitution.

Disposal: To avoid the contamination or propagation of any hazardous microbes the used, unusable or modified preparation of this product must be disposed after autoclaving after completion of task.

Reference

1. American Public Health Association, 1981, Standard Methods for the Examination of Water and Wastewater, 15th ed., APHA Inc., Washington, D.C.
2. Atlas, R. M. (2005). *Handbook of media for environmental microbiology*. CRC press.
3. Bureau of Indian Standards, IS:5887 (Part II) 1976, reaffirmed 1986
4. Difco Manual (1998). 11th Edition. Difco Laboratories., Division of Becton Dickinson and Company, Sparks, Maryland, USA.
5. MacFaddin J., (1985), *Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria*, Vol. 1, Williams and Wilkins, Baltimore.

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