Simmons Citrate Agar

Intended Use
Simmons Citrate Agar is used for the differentiation of Gram-negative bacteria on the basis of citrate utilization.

Summary
Simmons Citrate Agar is recommended by APHA and is used for the differentiation of *Enterobacteriaceae* and members of the *aerogenes* group on the basis of citrate utilization. Kosher developed a liquid medium containing ammonium salt as the only source of nitrogen, and citrate as the only source of carbon to differentiate between *Escherichia coli* and *Klebsiella aerogenes* based on the IMViC reactions. Simmons later on, modified this medium with the addition of agar and bromothymol blue. Organisms capable of utilizing citrate grow well on this medium. Simmons Citrate Agar is included in the Bacteriological Analytical Manual for food and cosmetics analysis.

Principle
Ammonium dihydrogen phosphate and sodium citrate serve as the sole nitrogen and carbon source respectively while bromothymol blue is the pH indicator. Organisms able to utilize the above compounds as sole source of nitrogen and carbon, grow on this medium and produce an alkaline reaction as indicated by the change in colour of bromothymol blue indicator from green (neutral) to blue (alkaline).

Formula*

Ingredients	**g/L**
Sodium Chloride	5.0
Sodium Citrate	2.0
Dipotassium Phosphate	1.0
Ammonium Dihydrogen Phosphate	1.0
Magnesium Sulphate	0.2
Bromothymol Blue	0.08
Agar	15.0
Final pH (at 25°C)	6.8 ± 0.2

*Adjusted to suit performance parameters.

Storage and Stability
Store dehydrated medium below 30ºC in tightly closed container and the prepared medium at 2ºC-8ºC. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Type of Specimen
Food samples

Specimen Collection and Handling
Ensure that all samples are properly labelled. Follow appropriate techniques for handling samples as per established guidelines. Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure. The samples must be stored and tested within the permissible time duration. After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions
1. Suspend 24.28 g of the powder in 1000 mL purified / distilled water and mix thoroughly.
2. Boil with frequent agitation to dissolve the powder completely.
3. Dispense in tubes or as desired.
4. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.
5. Cool in slanted position for use as slants.

Quality Control
Dehydrated Appearance: Yellow to turmeric yellow coloured, homogenous, free flowing powder.
Prepared Appearance: Forest green coloured, slightly opalescent gel forms in tubes as slants.
Cultural Response: Cultural characteristics observed after an incubation of 18-24 hours at 30°C-35°C.

Organism (ATCC)	Growth	Colour of Medium	Citrate Utilization
Klebsiella aerogenes (13048)	Good	Blue	+
Klebsiella pneumoniae subsp. *pneumoniae* (10031)	Good	Blue	+
Salmonella enterica subsp. *enterica* serovar *Typhimurium* (14028)	Good	Blue	+
Escherichia coli (25922)	Inhibited	Green	-
Shigella flexneri serotype 2b (12022)	Inhibited	Green	-

Interpretation of Results

1. A positive reaction (citrate utilization) is indicated by growth with an intense blue colour in the slant. A negative reaction is evidenced by no growth to slight growth with no change in colour of the medium.
2. *E. coli* including different serotypes from epidemic infantile enteritis, as well as *Shigella, Yersinia* and *Edwardsiella* species do not grow on this medium. *Serratia* and the majority of *Citrobacter*, *Klebsiella, Proteus* and *Providencia* species, except *Morganella morganii* and *Klebsiella hinoscleromatis* utilize citrate and produce the characteristic blue colouration.
3. This medium may also be able to differentiate citrate positive *Salmonella enteritidis* and members of *Salmonella* subgenus 2, 3 and 5 from the citrate negative *S. Typhi, S. paratyphi* A*, S. pulloram* and *S. gallinarum.*

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Precautions / Limitations

1. Do not carry over any nutrients into the medium as it may lead to false positive results.
2. Dilute the inoculum before inoculating the medium to avoid a carryover of other carbon sources.
3. Use a light inoculum while streaking.

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. Downes and Ito (ed.) 2001, Compendium of Methods for The Microbiological Examination Of Foods, 4th edition, APHA Washington DC.
2. H. Wehr and J. Frank, 2004, Std. Methods for The Examination of Dairy Products, 17th Edition; APHA, Washington, DC.
3. Koser, 1923, J. Bact; 8:493.
4. Simmons, 1926, J. Infec. Dis; 39: 209.
5. US Food and Drug Adm; 1998, Bacteriological Analytical Manual, 8th Ed; Rev. A, AOAC, International, Gaithersburg, Md.
6. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201190170100	Dehydrated Culture Media	100 g
201190170500	Dehydrated Culture Media	500 g
201190172500	Dehydrated Culture Media	2.5 k
203190540012	Ready Prepared Slant	12 Slants