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## SIMMONS CITRATE AGAR

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### INTENDED USE

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

### SUMMARY AND EXPLANATION

Koser,<sup>1</sup> in 1923, developed a liquid medium consisting of inorganic salts in which an ammonium salt was the only source of nitrogen and citrate was the sole carbon source in order to differentiate between what are now known as *Escherichia coli* and *Enterobacter aerogenes* as part of the IMViC (Indole- Methyl Red- Voges Proskauer-Citrate) reactions. Simmons,<sup>2</sup> in 1926, modified Koser's formulation with the addition of 1.5% agar and bromthymol blue.<sup>3</sup> Organisms capable of metabolizing citrate grow well on this medium.

### PRINCIPLE

Organisms able to utilize ammonium dihydrogen phosphate and sodium citrate as the sole sources of nitrogen and carbon, respectively, will grow on this medium and produce an alkaline reaction as evidenced by a change in the color of the bromthymol blue indicator from green (neutral) to blue (alkaline).

### REAGENTS (FORMULA)

Ammonium Dihydrogen Phosphate .....	1.0	g
Dipotassium Phosphate .....	1.0	g
Sodium Chloride .....	5.0	g
Sodium Citrate .....	2.0	g
Magnesium Sulfate .....	0.2	g
Bromthymol Blue .....	0.08	g
Agar .....	15.0	g
Deionized Water .....	1000.0	ml

### PROCEDURE

Inoculate slants with growth from a pure culture using a light inoculum. Incubate all tubes for 4 days at 35 ± 2°C in an aerobic atmosphere.

## EXPECTED RESULTS

A positive reaction is indicated by growth with an intense blue color in the slant. A negative reaction is evidenced by no growth to trace growth with no change in color (medium remains dark green).

Consult appropriate texts for additional differentiating characteristics.<sup>4,5</sup>

## QUALITY CONTROL

All lot numbers have been tested and have been found to be acceptable. Customers can test products using the following quality control organisms. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, sample results should not be reported.

Organisms	Incubation	Results
<i>Escherichia coli</i> ATCC 25922	35 ± 2°C for 18-24 hours	Growth, Alkaline (Blue)
<i>Klebsiella pneumoniae</i> ATCC 12022	35 ± 2°C for 18-24 hours	Growth, Alkaline (Blue)

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## BIBLIOGRAPHY

1. Koser. 1923. J. Bacteriol. 8:493.
2. Simmons. 1926. J. Infect. Dis. 39:209.
3. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
4. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
5. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

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