Simmons Citrate Agar

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

Summary and Explanation
Koser,[1] 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,[2] in 1926, modified Koser's formulation with the addition of 1.5% agar and bromthymol blue.[3] Organisms capable of metabolizing citrate grow well on this medium.

Principles of the Procedure
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).

Formula
BBL™ Simmons Citrate Agar

Approximate Formula* Per Liter		
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
Agar	15.0	g
Bromthymol Blue	0.08	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product
1. Suspend 24.2 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense and autoclave at 121°C for 15 minutes.
4. Allow to cool in a slanted position for use as slants. The agar also may be used as a plating medium.
5. Test samples of the finished product for performance using stable, typical control cultures.

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.[4,5]

References

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.

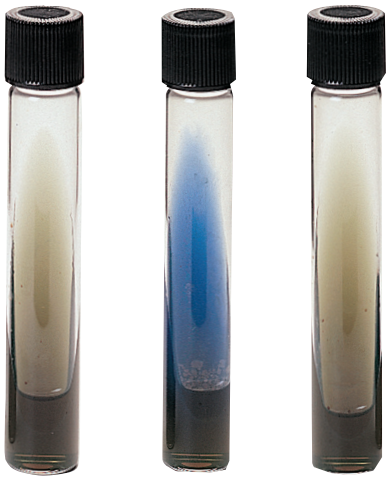
User Quality Control
Identity Specifications
BBL™ Simmons Citrate Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material, may contain many dark and gray flecks.
Solution:	2.42% solution, soluble in purified water upon boiling. Solution is medium to dark, green, clear to slightly hazy.
Prepared Appearance:	Medium to dark, green, clear to slightly hazy, with a small amount of precipitate and as many as a large amount of insolubles.
Reaction of 2.42% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response
BBL™ Simmons Citrate Agar

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 35 ± 2°C for 4 days.

ORGANISM	ATCC™	RECOVERY	REACTION
Enterobacter aerogenes	13048	Good	Alkaline (blue)
Escherichia coli	25922	Partial to complete inhibition	–
Klebsiella pneumoniae	33495	Good	Alkaline (blue)
Shigella flexneri	9199	Complete inhibition	–



Uninoculated Tube | *Enterobacter aerogenes* ATCC™ 13048 | *Escherichia coli* ATCC™ 25922

Availability

BBL™ Simmons Citrate Agar

AOAC BAM CCAM COMPF ISO

Cat. No.	211620	Dehydrated – 500 g
	221026	Prepared Slants – Pkg. of 10*
	221027	Prepared Slants – Ctn. of 100*

**Store at 2-8°C.*