

Simmons Citrate Agar

For the determination of citrate utilization by enterobacteria

Formula in grams per liter:

Ammonium Dihydrogen Phosphate	1,00	Dipotassium Phosphate	1,00
Sodium Chloride	5,00	Sodium Citrate	2,00
Magnesium Sulfate	0,20	Bacteriological Agar	15,00
Bromothymol Blue	0,08		

Final pH: 6,9 ± 0,2 at 25 °C

Preparation:

Suspend 24,3 grams of the medium in one liter of distilled water. Mix well and heat with frequent agitation until completely dissolved. Dispense in tubes and sterilize in the autoclave at 121°C (15 lbs sp.) for 15 minutes. Cool the tubes in a slanted position so that the base is short (1-1,5 cm. deep). Alternatively, the media can be poured into petri plates.

Uses:

Simmons Citrate Agar is used to differentiate enteric Gram-negative bacilli on the basis of sodium citrate utilization as a source of carbon and inorganic ammonium salt utilization as a source of nitrogen. It is recommended for the differentiation of coliforms isolated from water. It is used in the same manner as Koser Citrate Broth for the utilization of citrate as one of the IMVIC reactions. It can be poured into plates or dispensed in tubes with long slants. The surface of the slant is inoculated and the base stabbed. The tubes are incubated at 35-37°C for 4 days. If good results are not obtained, as in the case of some *Providencia* strains, incubate for 7 days. Only those organisms capable of utilizing citrate as a source of carbon grow on the slant and produce a color change from green to blue (alkaline).

This medium can be used especially for the differentiation of enteric organisms as follows:

NEGATIVE	POSITIVE	
<i>Escherichia</i>	<i>Arizona</i>	<i>Enterobacter</i>
<i>Shigella</i>	<i>Citrobacter</i>	<i>Klebsiella</i>
<i>S. typhi</i>	<i>S. paratyphi B</i>	<i>Serratia</i>
<i>S. paratyphi A</i>	<i>S. typhimurium</i>	

Microbiological Tests:

Microorganisms	Growth	Medium color
<i>Enterobacter aerogenes</i> ATCC 13048	Good	Blue
<i>Escherichia coli</i> ATCC 25922	Inhibited	Green
<i>Salmonella enteritidis</i> ATCC 13076	Good	Blue
<i>Shigella dysenteriae</i> ATCC 13313	Inhibited	Green
<i>Salmonella typhimurium</i> ATCC 14028	Good	Blue
<i>Salmonella typhi</i> ATCC 19430	Good	Green

