SIMMONS CITRATE AGAR

- For in vitro use only - Catalogue No. PS60

Our Simmons Citrate Agar is used for the differentiation of *Enterobacteriaceae* based on citrate utilization.

Simmons Citrate Agar is based on the work of Koser who first developed a liquid medium for differentiating coliforms from fecal coliforms based on the utilization of citrate as the sole source of carbon. The Koser medium required additional testing, as the uninoculated medium appeared turbid. Simmons improved upon the Koser medium be adding agar and the pH indicator bromothymol blue.

Sodium citrate is the carbon source in the medium and differentiates those organisms able to utilize citrate as its sole source of carbon. Ammonium dihydrogen phosphate is the sole source of nitrogen and its utilization results in an alkaline shift in the pH of the medium detectable by a green to blue color shift in the pH indicator, bromothymol blue. Sodium chloride provides an isotonic growth environment for bacteria while dipotassium phosphate acts as a buffering agent to maintain a stable pH.

Simmons Citrate Agar is primarily used to aid in the identification of *Enterobacteriaceae.* Uses include:

1. *Escherichia coli* (usually –) and *Shigella* spp. (–) from other commonly encountered *Enterobacteriaceae* (variable +)
2. *Edwardsiella* spp. (–) from *Salmonella* spp. (usually +)
3. *Serratia proteamaculans* (+) from *Yersinia pseudotuberculosis* (–) and *Yersinia enterocolitica* (usually –)
4. *Klebsiella-Enterobacter* groups (usually +) from *E. coli* (usually –)
5. *Proteus rettgeri* (+) from *Morganella morganii* biogroups 1 and 2 (–)
6. *Yokenella regensburgei* (+) from *Hafnia alvei* (–)
7. *Leminorella grimontii* (+) from *L. richardii* (–)
8. *Acidovorax delafieldii* (+) from *A. facilis* (–) and *A. temperans* (–)

Formulation per Litre of Medium

Magnesium Sulfate	0.2 g
Ammonium Dihydrogen Phosphate	1.0 g
Dipotassium Phosphate	1.0 g
Sodium Citrate	2.0 g
Sodium Chloride	5.0 g
Agar	15.0 g
Bromothymol Blue	0.08 g

pH 6.8 ± 0.2

Recommended Procedure

1. Allow medium to reach room temperature prior to inoculation.
2. Using a pure, direct inoculum, perform a four-quadrant streak to obtain well-isolated colonies.
3. Incubate aerobically at 35°C.
4. Examine after 24 and 48 hours.

Interpretation of Results

Positive (+): Growth accompanied by a blue color change on the agar surface

Negative (–): No growth or poor growth with no color change (remains green)

Additional tests should be performed on isolated colonies from pure culture in order to complete identification.

- *Some citrate-positive organisms may require 48 hours or longer for a discernable positive color reaction*

- *During inoculation, nutritional carry-over from other media can result in false-positive results. Ensure to flame the inoculating needle or loop prior to inoculation; dilution of the sample in saline prior to inoculation can also minimize such occurrences*

- *The inoculum should be from a pure, overnight culture grown on a solid medium and not from a broth suspension*

Quality Control

After checking for correct pH, colour, depth, and sterility, the following organisms are used to determine the growth performance of the completed medium.

Organism	Expected Result
Enterobacter aerogenes ATCC 13048	Good growth with blue color change
Escherichia coli ATCC 25922	Poor or no growth with no color change

Storage and Shelf Life

Our Simmons Citrate Agar should be stored away from direct light at 4 to 8°C. The medium side should be uppermost to prevent excessive accumulation of moisture on the agar surface. Under these conditions the medium has a shelf life of 12 weeks from the date of manufacture.

Ordering Information

Cat#	Description	Format
PS60	Simmons Citrate Agar [Standard 15x100-mm plate]	10/pkg

References

1. Koser SA. Utilization of the salts of organic acids by the colon-aerogenes group. J Bacteriol 1923; 8:493-520.
2. Simmons JS. A culture medium for differentiating organisms of typhoid-colon aerogenes groups and for isolation of certain fungi. J Infect Dis 1926; 39:209-14.
3. Vaughn RH, Osborne JT, Wedding GT, et al. The utilization of citrate by *Escherichia coli*. J Bacteriol 1950; 60;119-27.
4. Branson D. Methods in clinical microbiology. Springfield, IL: Charles C Thomas, 1972.
5. MacFaddin JF. Media for isolation-cultivation-maintenance of medical bacteria, Vol I. Baltimore: Williams & Wilkins, 1985.
6. Isenberg HD, Ed. Clinical microbiology procedures handbook. Washington, DC: ASM, 1992.
7. MacFaddin JF. Biochemical tests for identification of medical bacteria. 3rd ed. Philadelphia: Lippincott Williams & Wilkins, 2000.

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