BIO-RAD

SIMMONS CITRATE

61834
64834

AGAR / MEDIUM FOR THE DIFFERENTIATION OF ENTEROBACTERIACEAE

IVD

1- INTENDED USE

Simmons citrate agar (sodium citrate minimum mineral medium) is used for the differentiation of Gram negative bacilli. It enables the detection of sodium citrate as the sole source of carbon and energy for bacteria. This medium contributes to demonstration of the identification characteristics of Enterobacteriaceae.

2- PRINCIPLE

Bacteria able to utilise sodium citrate as sole carbon source are able to grow on this medium. Fermentation of sodium citrate induces acidification, causing a **blue** colour change of the medium in the presence of bromothymol blue (pH indicator).

3- HOW SUPPLIED

- Ready to use medium:
  - 25 x 7 ml slant tubes code 61834
- Dehydrated medium
  - bottle of 500 g code 64834

4- THEORETICAL COMPOSITION (g/l of distilled water)

Simmons citrate agar is prepared according to the formula described by Simmons (1)

Sodium citrate	1
Sodium chloride	5
Magnesium sulphate	0.2
Ammonium dihydrogen phosphate	1
Dipotassium phosphate	1
Bromothymol blue	0.08
Agar	15
Final pH	6.8 ± 0.2

Preparation of the medium:
Homogenize the powder contained in the bottle.
Add **23 grams** of dehydrated medium to 1 litre of sterile distilled water. Heat gently, shaking frequently, then heat to boiling for 1 minute. Sterilize in the autoclave at 121°C for 15 minutes. Dispense into tubes or bottles. Cool the tubes in an inclined position to obtain a long slope without a mass of agar in the bottom of the tube.

5- STORAGE

- Ready to use medium: at +2-8°C in a dark place.
- Dehydrated medium: tightly sealed bottle in a dry place at +15-25°C.

The expiry date and batch number are indicated on the packaging.

6- INSTRUCTIONS

Material:
- Material provided: Simmons Citrate medium

Inoculation:
This medium must be inoculated with a pure, fresh culture of Enterobacteriaceae grown on agar medium. A culture in broth or peptone water must never be used, as these media would provide other nutrients that may lead to erroneous results.
Inoculate on the surface, by a central, longitudinal streak.

Incubation:
Incubate in the incubator at 37°C for 1 to 7 days.

Reading:
Observe the culture daily.
"Citrate-positive" bacteria turn this medium **blue** and often show abundant growth.
"Citrate-negative" bacteria do not grow on this medium and do not turn the medium blue, even after several days of incubation.
(+): positive in the majority of cases.

Citrate +	Citrate –	Citrate-variable (depending on the biochemical type
Salmonella subspecies 1 (in general) *S. arizonae* BE II *Citrobacter*	*S. paratyphi* A *S. typhi* *Edwardsiella*	*S. paratyphi* B: (+) *S. typhimurium*: (+) *S. enteritidis*: (+)
Levinea	*Escherichia coli* *E. coli* biotype A.D. *Shigella*	
P. rettgeri *Providencia*	*P. morganii*	*P. mirabilis* *P. vulgaris*
K. pneumoniae *K. oxytoca* *Enterobacter aerogenes* *E. cloacae* *E. agglomerans* (in general) *Serratia*	*K. rhinoscleromatis* *Hafnia alvei* (late utilisation at 22-30°C)	*K. ozaenae*
Vibrio cholerae	*Yersinia enterocolitica* *Yersinia pseudotuberculosis* (except serotype 4) *Plesiomonas*	*Aeromonas hydrophila* *Vibrio NAG*
P. aeruginosa *Pseudomonas* sp.	*X. maltophilia* *Pasteurella*	*Acinetobacter*

7- PERFORMANCE/QUALITY CONTROL OF THE TEST

- Appearance of the ready to use medium: clear **green** agar.
- Appearance of the dehydrated medium: **greenish** powder.
- The growth performances of Simmons Citrate medium are verified with the following strains:

STRAINS	CULTURE RESULT AFTER 1 to 7 DAYS at 37°C
Salmonella Enteritidis ATCC 13076	Positive
Klebsiella pneumoniae ATCC 13883	Positive
Proteus vulgaris ATCC 13315	Negative
Escherichia coli ATCC 25922	Negative

8- QUALITY CONTROL OF THE MANUFACTURER

All manufactured reagents are prepared according to our Quality System, starting from reception of raw material to the final commercialization of the product. Each lot is submitted to quality control assessments and is only released to the market, after conforming to pre-defined acceptance criteria. The records relating to production and control of each single lot are kept within Bio-Rad.

9- LIMITS OF USE

- It is very important not to add any other nutrients to the medium during inoculation, as any additional nutrients can lead to erroneous results.
- Certain “citrate-positive” bacteria may take several days to turn the medium blue.
- Complementary tests must be performed to identify the species of the strain isolated.
- Pure, fresh cultures must always be used to obtain interpretable results.

10- REFERENCES

1. SIMMONS, J.S. 1926. A culture medium for differenciating organisms of typhoid-colon aerogenes groups and for isolation of certain fungi. J. Infect. Dis., 1926, **39** : 209.

Bio-Rad
3, boulevard Raymond Poincaré
92430 Marnes-la-Coquette France
Tel. : +33 (0) 1 47 95 60 00
Fax : +33 (0) 1 47 41 91 33

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11/2005