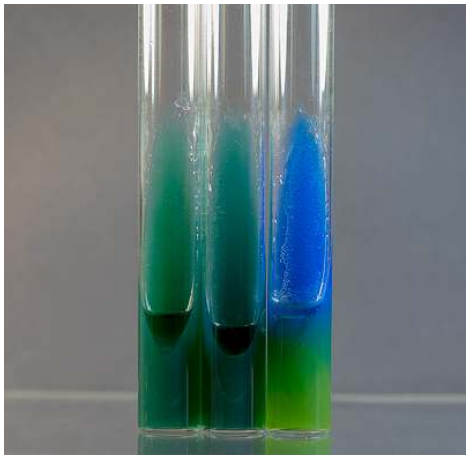
INSTRUCTIONS FOR USE

SIMMONS CITRATE AGAR

Dehydrated culture medium



Simmons Citrate Agar-from left:
uninoculated tube, *E.coli*, *E. aerogenes*

1 - INTENDED USE

In vitro diagnostic device. Medium for the differentiation of *Enterobacteriaceae* based on the utilization of citrate as the sole source of carbon.

2 - COMPOSITION -TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Ammonium dihydrogen phosphate	0.80 g
Sodium ammonium phosphate	0.80 g
Sodium chloride	5.00 g
Sodium citrate	2.00 g
Magnesium sulphate	0.20 g
Bromothymol blue	0.08 g
Agar	15.00 g

*the formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Simmons Citrate Agar is a modification developed by Simmons[1] of the liquid medium of Koser[2], with the addition of 1.5% agar and bromothymol blue as pH indicator.[3]
In Simmons Citrate Agar the sole source of nitrogen is sodium ammonium phosphate and the sole source of carbon is sodium citrate. The metabolism of citrate is based on the enzyme citrate-permease, which requires for its activation the presence of divalent cations, given in the medium by magnesium sulphate. The result of citrate metabolism is the formation, in an acid environment, of acetate, lactate, acetoin, carbonates and bicarbonates. The degradation of inorganic ammonium salts results in the formation of ammonia. Bacteria that utilize citrate as the sole source of carbon, utilize ammonium salt as the sole source of nitrogen and grow with alkalinisation of the medium, indicated by the colour change of bromothymol blue from green to blue.
Simmons Citrate Agar may be used to differentiate *E.coli* (citrate negative) from *E.aerogenes* (citrate positive), and citrate-positive Salmonella Enteritidis and members of *Salmonella* subgenera II, III and IV from Salmonella Typhi, Salmonella Paratyphi A, Salmonella Pullorum and Salmonella Gallinarum (citrate negative).
Citrate test is recommended by ISO 10273 for differentiating *Y. enterocolitica* (citrate negative) from other species of the genus *Yersinia* such as *Y. intermedia, Y. frederiksenii, Y. rohdei, Y. aldovae* (citrate positive or variable).[4]

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 23,2 g in 1000 mL of cold purified water, heat to boiling with frequent agitation, distribute in tubes and autoclave at 121°C for 15 minutes. Cool in slanted position (long slant/short butt). All glassware must be chemically clean and alkali free. The medium can be used for citrate test in Petri dishes too.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	dark yellow, fine, homogeneous, free-flowing powder
Solution and tubed medium appearance	green, limpid
Final pH at 20-25 °C	7.0 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Simmons Citrate Agar CND: W0104010101; EDMA:14.01.01.01; RDM1875716/R	Dehydrated medium	4020452	500 g (20.7)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops, incubator and laboratory equipment as required, Erlenmeyer flasks, screwcap tubes, ancillary culture media and reagents for the identification of the colonies.

8 - SPECIMENS

This medium is not intended to be used for primary isolation; it must be inoculated with pure cultures of organisms isolated from clinical and non clinical specimens.

9 - TEST PROCEDURE

The medium may be used either as slopes in test tubes or as a plate medium in Petri dishes. In both cases the surface of the medium must be lightly inoculated by streaking and, where slopes are used, the butt of medium is inoculated by stabbing. Incubate in aerobic atmosphere with the loose caps at 35-37°C and record the results after 24-48 hours. Some citrate positive bacteria require more than 48 hours to develop the reaction: in case of a negative result, incubate up to 4 days.

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10 - READING AND INTERPRETATION

After incubation, observe the growth and the colour change of the medium.
Citrate positive: growth with intense blue colour on the slant (alkalinity).
Citrate negative: no growth with no change in colour.

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, it is responsibility of the end-user to perform Quality Control testing in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.
Citrate positive test-strain: *E.aerogenes* ATCC 13048
Citrate negative test-strain: *E.coli* ATCC 25922
Incubation in aerobic atmosphere at 35-37°C for 18-24 hours.

ATCC is a trademark of American Type Culture Collection

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of dehydrated Simmons Citrate Agar is tested for specific performance characteristics (citrate test) by comparing the results with a previously approved Reference Batch. Samples are inoculated directly by streaking and stabbing the tubed medium with pure cultures of *E.coli* ATCC 25922, *E.aerogenes* ATCC 13048, *K.pneumoniae* ATCC 27736, *P.stuardii* ATCC 33672, *S.flexneri* ATCC 12022, S.Typhimurium ATCC 14028, S.Gallinarum clinical isolate, *Y.enterocolitica* ATCC 23715. Tubes are incubated with loose caps at 35–37 °C for 18-24 hours in an aerobic atmosphere. The colour changes of tubed medium are observed and recorded. Citrate positive with growth and blue colour: *E.aerogenes, K.pneumoniae, P.stuardii*, S.Typhimurium; citrate negative, no growth and no colour change: *E.coli, S.flexneri*, S.Gallinarum, *Y.enterocolitica.*

13 - LIMITATIONS OF THE METHOD

- If a large inoculum is used to streak, the slant may give a false positive result.[3]
- Be careful not to remove traces of culture medium together with the colonies to be tested as this can lead to false positives. Some authors recommend diluting the inoculums in saline prior to inoculation of the medium to avoid a carry-over of other carbon sources.[3]
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification. If relevant, perform antimicrobial susceptibility testing.
- This culture medium is intended as an aid in the diagnosis of infectious disease; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

14 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Material Safety Data Sheet.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the inoculated tubes or plates with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption
- The Certificates of Analysis and the Safety Data Sheet are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use the container must be tightly closed. Discard the product if the container and/or the cap were damaged or in case of evident deterioration of the powder (colour changes, hardening, large lumps).

16 - REFERENCES

1. 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
2. Koser SA 1923. Utilization of the salts of organic acids by the colon-aerogenes group. J Bacteriol 1923; 8:493
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
4. ISO 10273:2017. Microbiology of the food chain — Horizontal method for the detection of pathogenic Yersinia enterocolitica

TABLE OF APPLICABLE SYMBOLS

REF o REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place

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REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2020/05

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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