ChromArt

SENECA

(**S**imultaneous Enumeration **En**terobacteriaceae **E**.**c**oli **A**gar)

Dehydrated culture medium and supplement

SENECA: *E. coli* blue colonies;
K. pneumoniae and S. Enteritidis (red colonies)

1 - Intended use

For the simultaneous enumeration of *Enterobacteriaceae* and *E. coli* in water, food and other samples of sanitary interest.

2 - Composition *

(after reconstitution with 1 L of water)

SENECA Base (dehydrated medium)

Peptones	15.00 g
Carbohydrates	2.50 g
Selective compounds	0.50 g
Phosphate buffer	4.30 g
Chromogenic mix	0.12 g
Agar	15.00 g

SENECA EE-EC Supplement (vial content for 500 mL of medium)

Antimicrobial compounds	4.5 mg
Chromogenic substrate	6.25 mg

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

3 - Principle of the method and explanation of the procedure

SENECA represents a development of the EE-EC Agar medium, which Biolife designed and proposed, first in the world, for the simultaneous enumeration of *E. coli* and *Enterobacteriaceae*.
Counting of *E. coli* alone is done by determination of the enzyme β-D-glucuronidase on SENECA Base.[1-3] Simultaneous enumeration of *Enterobacteriaceae* and *E. coli* is done by addition of a specific supplement (SENECA EE-EC Supplement) to the base medium that provides the *Enterobacteriaceae* colonies with a pink-red coloration.

4- Directions for medium preparation

Suspend 18.7 g of SENECA Base in 500 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 min. Cool to 47-50°C.
For *E. coli* enumeration: use as is.
For simultaneous enumeration of *E. coli* and *Enterobacteriaceae*: dissolve the contents of one vial of SENECA EE-EC Supplement (REF 4240023) with 1 mL of ethanol, mix and then add 1 mL of sterile distilled water; add to 500 mL of autoclaved and cooled to 47-50° SENECA Base.

5 - Physical characteristics

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared plates appearance	dark yellow, limpid
Freeze-dried supplement	pink pellet; pale yellow and clear solution with a slight precipitate after reconstitution
Final pH of complete medium (at 20-25°C)	7.3 ± 0.2

6 - Materials provided – packaging

Product	Type	REF	Pack
SENECA Base	Dehydrated medium	405582S2	500 g (13.36 L)
SENECA EE-EC Supplement	Freeze-dried supplement	4240023	10 vials, each for 500 mL of medium

7 - Materials required but not provided

Autoclave, water-bath, sterile loops, swabs and pipettes, incubator and laboratory equipment as required, Petri dishes, Erlenmeyer flasks, ancillary culture media and reagents.

8 - Specimens

Water, food, feed, food chain samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable international standards.

9 - Test procedure

Membrane filtration method
Filter 100 mL (or other volumes, e.g., 250 mL for bottled water) of the sample using a membrane filter usually about 47 mm or 50 mm in diameter, with filtration characteristics equivalent to a rated nominal pore diameter of 0,45 µm and, preferentially, with grid lines. The minimum volume for filtration is 10 mL of sample or dilutions thereof to ensure even distribution of the bacteria on the membrane filter.
After filtration place the membrane filter on the medium, ensuring that no air is trapped underneath
Pour-plate method
Pour 1 mL of the initial suspension and decimal dilutions of the sample into the plates. Add about 15 mL of pre-cooled medium. Mix well the inoculum with the medium.
Surface plating technique
1.Dry the prepared plates before the use.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it

2. Using a sterile pipette, transfer 0.1 mL of the test sample, if the product is liquid, or of the initial suspension in the case of other products, to the centre of a plate.
3. Carefully spread the inoculum uniformly and as quickly as possible over the surface of the agar plate, without touching the sides of the dish with the spreader.
4. Leave the plates with the lids on for about 15 min at ambient temperature for the inoculum to be absorbed into the agar.

Incubation

Incubate for 24 ± 2 h at 37°± 1 C. In case of slight growth, poor pigmentation or no growth, re-incubate for additional 24 h.

10 - READING AND INTERPRETATION

After incubation, observe bacterial growth, record each specific color and morphological characteristic of the colonies.
Enumeration of *E. coli* + *Enterobacteriaceae* (SENECA Base 405582S + SENECA EE-EC Supplement 4240023).
Count as *Enterobacteriaceae* all red and blue colonies.
Count as *E. coli* β-D glucuronidase positive all blue colonies.
Enumeration of *E. coli* (SENECA Base 405582S)**.**
Count as *E. coli* β-D glucuronidase positive all blue colonies.

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, the end user can perform its own Quality Control 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.

Productivity control - *E. coli* ATCC 25922: growth, blue colonies
Specificity control - *E. aerogenes* ATCC 13048: growth, red colonies
Selectivity control - *S. aureus* ATCC 25923: inhibited; *P. aeruginosa* ATCC 14207: inhibited

ATCC is a trademark of American Type Culture Collection

12- PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of SENECA Base supplemented with SENECA EE-EC Supplement (TB:Test Batch) is assessed for productivity, specificity and selectivity by comparing the results with a previously approved Reference Batch (RB).
Productivity and specificity are tested by a quantitative test with the target strains *E. coli* ATCC 25922 and S.Typhimurium ATCC 14028: the plates are inoculated with decimal dilutions in saline of a colonies' suspension and incubated at 37°C for 24 hours. The colonies are enumerated on both batches and the productivity ratio ($Pr:CFU_{RB}/CFU_{TB}$) is calculated. If *Pr* is ≥ 0.7 and if the colonies morphology and colour are typical (*E. coli* blue colonies, S. Typhimurium red colonies) the results are considered acceptable and conform to the specifications. Furthermore the productivity characteristics are tested by semi-quantitative ecometric technique with the following target strains: *C.freundii* ATCC 8090, *P.mirabilis* ATCC 12453, *P.stuardii* ATCC 33672. The amount of growth and colonies characteristics are evaluated after incubation at 37°C for 24-48 hours: all test strains exhibit good growth with typical red colonies.
Selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the non-target strains *P. aeruginosa* ATCC 27853, *A. hydrophila* ATCC 7965. *E. faecalis* ATCC 19433, *S. aureus* ATCC 25923, *B. subtilis* ATCC 6633. After incubation at 37°C for 24hours, the growth of non-target strains is totally inhibited.

13 - LIMITATIONS OF THE METHOD

- Approximately 3-4% of *E. coli* are β-glucuronidase negative, notably *E. coli* O157 strains.[4] Consequently, some strains of *E. coli*, including pathogenic ones, will not be detected on SENECA Base and will be recognized as *Enterobacteriaceae* on SENECA EE-EC.
- It has been reported that approximately 40% of *Shigella* species, various bio-serotypes of *Salmonella* (13% of *Salmonella* subgenus I) may be β-glucuronidase positive; only exceptionally this test is positive with *Providencia, Enterobacter* and *Yersinia* strains (1-5%).[5-6]
- If heavy contamination is suspected, in the pour plate technique, after inoculation of the base layer and its solidification, add a surface layer of about 5 mL of the same medium.

14 - PRECAUTIONS AND WARNINGS

- The medium base and the supplement are for microbiological control and for professional use only; they are to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- The medium base and the supplement shall be used in association according to the described directions. Apply Good Manufacturing Practice in the production process of prepared media.
- Dehydrated media and antibiotics containing supplements must be handled with suitable protection. Before use, consult the Material Safety Data Sheets.
- This culture medium contains raw materials of animal origin. The ante and post mortem controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that the product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Be careful when opening the metal ring to avoid injury.
- The supplement is sterilized by membrane filtration.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplement or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and supplement and the sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium and the supplements as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheets 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

Dehydrated medium

Upon receipt, store at +2°C /+8°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 are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).

Freeze-dried supplement

Upon receipt, store the product in the original package at +2°C /+8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once the vial has been opened and the lyophilised product has been reconstituted, the resulting solution should be used immediately. Before use, examine the lyophilized and reconstituted product and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).

The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type (plates/flasks) and the applied storage conditions (temperature and packaging).

16 - REFERENCES

1. Bascomb S. Enzyme tests in bacterial identification. Methods Microbiol 1987; 19:105-160.
2. Manafi M, Kneifel W, Bascomb S Fluorogenic and chromogenic substrates used in bacterial diagnostics. Micr Rev, Sept. 1991, p. 335-348
3. Watson RR.. Substrate specificities of aminopeptidases:a specific method for microbial differentiation, p. 1-14. In J. R.Norris (ed.), Methods in microbiology, vol. 9. 1976, Academic Press (London), Ltd., London.
4. Feng P, Lampel KA, Karch H, Whittam TS. Genotypic and phenotypic changes in the emergence of Escherichia coli O157:H7. J. Infect. Dis. 177: 1750–1753.
5. Kilian M. & Bulow P. Rapid diagnosis of Enterobacteriaceae. Detection of bacterial glycosidases. Acta Pathol Microbiol Scand Sect B. 1976, 84: 245–251.
6. Le Minor, Buissière J, Novel G, Novel M. Relation entre le sérotype et l'activité β-glucuronidasique chez les Salmonella. Ann Microbiol (Paris) 1978; 129B (2) :155–165.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	This side up	Store in a dry place	Fragile
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	

REVISION HISTORY

Version	Description of changes	Date
Revision 2	Updated layout and content	2023/02

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

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it