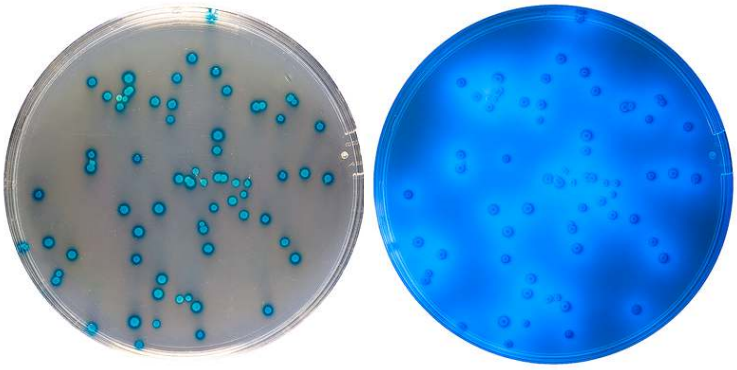
ChromArt

C-EC AGAR

Dehydrated and ready-to-use culture medium



On the left: *E.coli* colonies in sunlight; on the right: the same plate under Wood's lamp.

1 - INTENDED USE

Chromogenic and fluorogenic medium for the simultaneous detection of coliforms and *Escherichia* coli in water.

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

Tryptose	10.00 g
Tryptophan	1.00 g
Peptocomplex	5.00 g
Yeast extract	3.00 g
Sodium chloride	5.00 g
Bile Salts n. 3	1.50 g
Isopropyl ß-D-1-thiogalactopyranoside (IPTG)	0.10 g
5-Bromo-4-chloro-3-indolyl beta-galactoside (X-GAL)	0.08 g
4-Metilumbelliferil-β-D-glucuronide (MUG)	0.05 g
Agar	13.00 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

Faecal pollution is the major cause of waterborne diseases, since most of the pathogens associated with transmission reside in human and warm-blooded animal faeces. Examination of samples of sanitary significance for the presence of *E.coli* and coliform bacteria provides an indication of such pollution.

C-EC Agar medium allows the simultaneous quantitative determination of total coliforms and *E. coli* with incubation at 37°C for 18-24 hours, or the detection of *E. coli* and faecal coliforms with incubation at 44°C. C-EC Agar is included in the APAT guidelines for water analysis.[1]

Tryptose and Peptocomplex provide nitrogen, carbon, amino acids and minerals for the microbial growth, yeast extract is a source of vitamins, particularly of group B. Sodium chloride maintains the osmotic balance. Sodium pyruvate stimulates a quick bacterial growth and aids in resuscitation of stressed cells. Bile salts no. 3 inhibit the growth of Gram-positive bacteria.

Detection of coliform bacteria is based on the ability of β-D-galactosidase to cleave the substrate X-GAL with the formation of blue-green colonies. Enumeration of *E. coli* is based on the detection of β-D-glucuronidase, in addition to β-D-galactosidase, which cleaves the fluorogenic substrate MUG, with the formation of dark blue colonies, fluorescent under Wood's lamp. The hydrolysis of X-GAL in enhanced by IPTG, a lactose operon inducer. The indole test can confirm the presence of *E. coli* by adding a drop of Kovacs' reagent to the colonies.

C-EC Agar was one of the first chromogenic culture media proposed in the early 1990s for microbial isolation and differentiation and has been the subject of published trials by Bonadonna et al.[2,3], Jermini et al.[4], Cesaroni et al.[5].

4A- DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION

Suspend 38.8 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation until completely dissolved. Sterilize in the autoclave at 121°C for 15 minutes. Cool to 47-50°C, mix well and dispense in sterile Petri dishes.

4B DIRECTIONS FOR BOTTLED MEDIUM PREPARATION

Liquefy the contents of the flask in an autoclave set at 100 ± 2°C or in a temperature-controlled water bath (100°C). Alternatively, the bottle may be placed into a jar containing water, which is placed on a hot plate and brought to boiling. Slightly loosen the cap before heating to allow pressure exchange. Cool to 47-50°C and pour the medium into sterile Petri dishes, under aseptic conditions.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared plates and flasks appearance	pale yellow, limpid or slightly opalescent
Final pH of complete medium (at 20-25°C)	7.4 ± 0.2

6 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
C-EC Agar	Dehydrated medium	4012982	500 g (12.9 L)
C-EC MF Plate	Ready-to-use plates	497101	3 x 10 plates ø 55 mm
C-EC Agar	Ready-to-use flasks	5112982	6 x 100 mL

7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

8 - SPECIMENS

Water samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards and norms.

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9 - TEST PROCEDURE

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 C-EC Agar, ensuring that no air is trapped underneath, invert petri dish, and incubate at 36 ± 1 °C for 18-24 h (*E. coli* and coliforms detection) or at 44 ± 1°C for 18-24 hours for *E. coli* and faecal coliforms detection.[1]

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies in natural light and under a Wood's lamp in semi-dark surroundings and count:
All blue-green colonies (positive for β-D-galactosidase reaction) as presumptive coliform or faecal coliform bacteria (depending on the incubation temperature).
All blue-green colonies (β-D-galactosidase positive) and fluorescent under Wood's lamp (β-D-glucuronidase positive) as *E. coli*.
Confirmation of *E. coli* identification can be performed by the indole test (+), directly on the plate with Kovacs' Reagent (REF 19171000).

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.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli ATCC 25922	37°C/ 18-24H/A	growth, blue-green colonies, fluorescent under Wood's lamp
E. aerogenes ATCC 13048	37°C/ 18-24H/A	growth, pink colonies, non-fluorescent under Wood's lamp
P. aeruginosa ATCC 27853	37°C/ 18-24H/A	growth, colourless colonies
E. faecalis ATCC 19433	37°C/ 18-24H/A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12-PERFORMANCES CHARACTERISTICS

Prior to release for sale representative samples of all lots of dehydrated and ready-to-use C-EC Agar are tested for productivity, specificity and selectivity by comparing the results with a previously approved Reference Batch.
Productivity is tested by a quantitative test with the target strains *E. coli* ATCC 25922, *E. coli* ATCC 8739: C-EC Agar plates are inoculated with decimal dilutions in saline of a suspension of colonies and incubated at 37°C for 24 hours. The colonies are enumerated on Test Batch (TB) and Reference Batch (RB) and the productivity ratio ($Pr=CFU_{TB}/CFU_{RB}$) is calculated. If Pr is ≥ 0.7 the results are considered acceptable and conform to the specifications. *E. coli* colonies are blue-green, fluorescent under Wood's lamp and indole positive.
Moreover, productivity is assessed by semi-quantitative ecometric technique with the following target strains: *C. freundii* ATCC 43864 and E. *aerogenes* ATCC 13048. After incubation, the amount of growth and the colony characteristics are evaluated: target strains exhibit good growth with blue-green colonies, non-fluorescent under Wood's lamp.
Specificity is tested by semi-quantitative technique with *P. aeruginosa* ATCC 27853. After incubation at 37°C for 24 hours *P. aeruginosa* grows with pale green/colourless 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 Gram positive strain *E. faecalis* ATCC 19433. The growth of non-target strain is totally inhibited.

13 - LIMITATIONS OF THE METHOD

- 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%).[6-8]
- Approximately 3-4% of *E. coli* are β-glucuronidase negative, notably *E. coli* O157 strains.[9,10] Consequently, these strains, being positive for β-galactosidase, will grow with blue-green colonies not fluorescent and be counted as coliforms.
- In addition to expressing β-D-glucuronidase, *E. coli* is able to produce indole from tryptophan. Therefore, in case of any doubt of *E. coli* colonies on the primary agar medium, indole test may be used as an additional confirmation.[9]
- To avoid false-positive results, caused by oxidase positive bacteria, for example, *Aeromonas* spp., the presumptive coliforms colonies shall be confirmed by a negative oxidase reaction (Oxidase Test Strips, REF 191040ST)
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

14 - PRECAUTIONS AND WARNINGS

- This culture medium is intended for microbiological control and 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 Safety Data Sheet.
- 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 this 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 in 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.
- Apply Good Manufacturing Practice in the production process of prepared media.
- Be careful when opening screw cap flasks to prevent injury due to breakage of glass.
- When using a hot plate and/or a water bath, boil sufficiently long to dissolve the whole medium.
- Wear heat-protective gloves during medium liquefaction. Do not place the hot flasks into an ice bath or in cold water to accelerate cooling as this might cause cracks in the glass.

- The time required for complete liquefaction of the medium may vary considerably and depends on the actual temperature of the heating device, its wattage, the size and volume of the bottle.
- Once the bottled medium is liquefied, it cannot be solidified and dissolved a second time.
- Each ready-to-use plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the inoculated 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 Sheets of the products 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).

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). According to APAT guidelines the self-prepared plates of C-EC Agar can be stored at 2-8°C for up to 2 weeks.[1]

Ready to use plates

Upon receipt, store plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g., microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

Ready to use flasks

Upon receipt, store flasks in their original pack at 2-8°C away from direct light. If properly stored, the flasks may be used up to the expiration date. Do not use the flasks beyond this date. Flasks from opened secondary packages can be used up to the expiration date. Opened flasks must be used immediately. Before use, check the closing and the integrity of the screw cap. Do not use flasks with signs of deterioration (e.g., microbial contamination, abnormal turbidity, precipitate, atypical colour).

16 - REFERENCES

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6. Trepeta RW, Edberg SC. Methylumbelliferyl- D-glucuronide-based medium for rapid isolation and identification of E. coli. J Clin Microbiol 1984; 19 :172.
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9. ISO 9308-1:2014 Water quality - Enumeration of Escherichia coli and coliform bacteria - Part 1: Membrane filtration method for waters with low bacterial background flora.
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TABLE OF APPLICABLE SYMBOLS

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

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/12

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

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