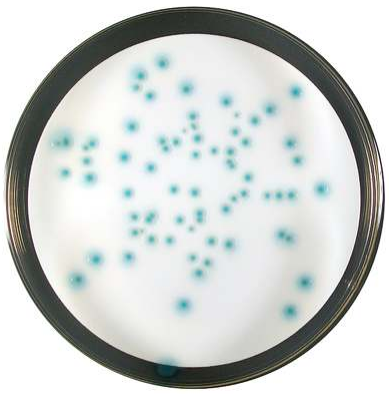
ChromArt

EC X-GLUC AGAR (CHROMOGENIC E. COLI)

Dehydrated and ready-to-use culture medium



EC X-GLUC Agar:
E.coli on a membrane filter

1 - INTENDED USE

Chromogenic medium for the enumeration of *Escherichia coli*

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

Tryptone	20.00
Yeast extract	5.00
Bile salts n. 3	1.50
Disodium hydrogen phosphate	5.00
Potassium dihydrogen phosphate	1.50
Sodium chloride	5.00
5-bromo-4-chloro-3-indolyl β-D-glucuronide (X-GLUC)	0.06
Tryptophan	1.00
Agar	12.00

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

EC X-GLUC Agar (Chromogenic E. coli), is a selective and differential medium for the enumeration and identification of *Escherichia coli*. The medium is included in the UNICHIM Standard No. 1185[1] for the detection of *E. coli* by the MF technique in water and in the review of methods for water ISSN:1125-2464[2].
Tryptone provides 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 while phosphates buffer the medium. Bile salts n° 3 act as a selective agent, inhibiting the growth of Gram-positive bacteria. Detection of *E. coli* is based on the ability of β-D-glucuronidase to cleave the substrate X-glucuronide with the formation of blue-green colonies.
Colonies cultured on EC X-GLUC Agar can be tested directly for indole by depositing a drop of Kovacs' reagent (REF 19171000) and observing for the red colour change of the reagent.
Natali et al.[3] evaluated EC X-GLUC Agar with microbial strains isolated from water samples and concluded that EC X-GLUC Agar gives better results than Levine EMB Agar and MacConkey Agar MUG in the detection of *E. coli*.

4A- DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION

Suspend 51 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C, mix well and pour into 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	beige, slightly opalescent
Final pH of complete medium (at 20-25°C)	7.0 ± 0.2

6 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
EC X-GLUC Agar	Dehydrated medium	4019682	500 g (9.8 L)
EC X-GLUC Agar	Ready-to-use plates	497102	3 x 10 plates ø 55 mm
EC X-GLUC Agar	Ready-to-use flasks	5119672	6 x 100 mL

7 - MATERIALS REQUIRED BUT NOT PROVIDED

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 and food 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.

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After filtration place the membrane filter on the EC X-GLUC Agar, ensuring that no air is trapped underneath, invert petri dish, and incubate at 44 ± 0.5 °C for 21-24 h.

Pour-plate method

Pour 1 mL of the decimal dilutions of the sample into the plates. Add about 15 mL of pre-cooled EC X-GLUC Agar. Mix well the inoculum with the medium. Incubate at 44 ± 0.5 °C for 21-24 h.

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Count all blue-green colonies (positive for β-D-glucuronidase) confirmed by indole test (+) as *E. coli*.

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:	44°C / 24 ore /A	growth with blue-green colonies, indole positive
E. aerogenes ATCC 13048	37°C / 24 ore /A	growth with colourless colonies, indole negative
E. faecalis ATCC 19433	37°C / 24 ore /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 EC X-GLUC Agar are tested for productivity, specificity and selectivity by comparing the results with a previously approved Reference Batch and with Tryptic Soy Agar.
Productivity is tested by a quantitative test with the target strains *E. coli* ATCC 25922 and *E. coli* ATCC 8739; EC X-GLUC Agar plates are inoculated with decimal dilutions in saline of a suspension of colonies and incubated at 44°C for 24 hours. The colonies are enumerated on Test Batch (TB) and on Tryptic Soy Agar (TSA) and the productivity ratio ($Pr=CFU_{TB}/CFU_{TSA}$) is calculated. If Pr is ≥ 0.5 and if the colonies' morphology and colour are typical (blue colonies, indole positive) the results are considered acceptable and conform to the specifications.
Specificity is tested by semi-quantitative ecometric technique with *C. freundii* ATCC 8090, *E. aerogenes* ATCC 13048 and *P. aeruginosa* ATCC 27853. After incubation at 37°C for 24 hours *P. aeruginosa* grows with pale green-beige colonies while *C. freundii* and *E. aerogenes* grow with colourless colonies, indole negative.
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%).[4-6]
- Approximately 3-4% of *E. coli* are β-glucuronidase negative, notably *E. coli* O157 strains.[6,7]
- 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.[7]

14 - PRECAUTIONS AND WARNINGS

- This culture medium is for Laboratory use 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 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.
- 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 sterilized medium inoculated 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 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).

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

1. Unichim n° 1185: 2000.
2. Bonadonna L. *Escherichia coli* nelle acque significato sanitario e metodologie di analisi. ISSN:1125-2464, 2001
3. Natali, P., Neri, A. Rossi, P., Ferrari, M. (1999) Biologi Italiani, n° 10/99, 20-22
4. Trepeta RW, Edberg SC. Methylumbelliferyl- D-glucuronide-based medium for rapid isolation and identification of E. coli. J Clin Microbiol 1984; 19 :172.
5. Robison BJ. Evaluation of a fluorogenic assay for detection of Escherichia coli in foods. Appl. Environ. Microbiol. 1984; 48:285-288
6. Kaluzewski SD, Tomczuk D. Evaluation of the Usefulness of Tests for Production of Beta-D-glucuronidase and Propylene Glycol Utilization for the Differentiation of Enterobacteriaceae Rods. Med Dosw Mikrobiol, 1995; 47:155-68.
7. 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.

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 4	Updated layout and content	2022/08

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

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