EC BROTH MUG

Dehydrated culture medium

1 - INTENDED USE

Selective medium for the confirmatory phase of procedures for detection and enumeration of *Escherichia coli* in foodstuffs and waters.

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

Tryptone	20.00 g
Lactose	5.00 g
Dipotassium hydrogen phosphate	4.00 g
Potassium dihydrogen phosphate	1.50 g
Sodium chloride	5.00 g
Bile salts n° 3	1.50 g
Tryptophan	1.00 g
4-methylumbelliferone beta-D-glucuronide (MUG)	0.05 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

Escherichia coli (EC) medium was first introduced by Hajna and Perry for improved selective detection of coliform bacteria and presumptive detection of *E. coli* in water, foods, shellfish, milk and other materials.[1,2] Feng and Hartman[3] developed EC medium with 4-methylumbelliferyl-β-D-glucuronide (MUG) for rapid screening of *E. coli* detection. Moberg[4] reported that a MUG concentration of 50 µg/mL provided the same intensity of blue fluorescence as the 100 µg/mL MUG levels. Koburger and Miller[5] recommended EC broth with MUG to test contamination in shellfish. Approved by the U.S. Environmental Protection Agency[6], EC-MUG is an effective and rapid method for detection and verification of *E. coli* in food, water, and environmental samples.[7]
EC Broth MUG is recommended by FDA-BAM[8] for the confirmatory MPN test for *E. coli* in shellfish meats and by APHA[9] for the confirmatory test of *E. coli* or thermotolerant coliforms and *E. coli* in water samples.
The EC Broth MUG tubes are inoculated with broth from positive presumptive tubes of Lauryl Sulphate Broth and incubated at 44.5 ± 0.2°C for 24 ± 2 h. If fluorescence is produced, the test is positive, indicating the presence of *E. coli*. The presence of thermotolerant coliforms and *E. coli* can be determined simultaneously by including a Durham tube in EC Broth test tubes.[9]
Tryptone provides nitrogen, carbon and minerals for microbial growth; lactose is a fermentable carbohydrate. Phosphates are used as buffering agents to control the pH in the medium. Sodium chloride maintains the osmotic balance. Bile salts n° 3, inhibit the development of Gram-positive bacteria, especially bacilli and enterococci, while at the same time promoting *E. coli* growth. MUG is cleaved by β-D-glucuronidase produced by *E. coli* to 4-methylumbelliferone and glucuronide; the fluorogenic 4-methylumbelliferone can be determined directly by using a long-wave ultraviolet light (Wood's lamp). Tryptophan is added to the medium for improving the performance of rapid direct indole test into the EC Broth MUG tubes.

4 - DIRECTIONS FOR MEDIUM PREPARATION

Suspend 38 g in 1000 mL of cold purified water; heat slightly to completely dissolve the powder, mix well and distribute 10 mL into test tubes containing inverted Durham tube. Sterilise by autoclaving at 121°C for 15 minutes. The Durham tubes shall not contain air bubbles after sterilization.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared tubes appearance	light yellow, limpid
Final pH at 20-25 °C	6.9 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
EC Broth MUG	Dehydrated medium	4014262	500 g (13.2 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, test-tubes, Durham tubes, ancillary culture media and reagents.

8 – SPECIMENS

Foods, waters, seawater and shellfish. Refer to applicable International Standards and regulations for the collection of samples. Operate in accordance with good laboratory practice for sample collection, storage and transport to the laboratory.

9 - TEST PROCEDURE

For the confirmatory test of *E. coli* proceed as following[8]:

1. From each of the incubated tubes with single strength and double-strength Lauryl Pepto Bios Broth (REF 401580) showing opacity, cloudiness or any visible gas inoculate with a sampling loop a tube of EC Broth MUG.
2. Incubate the tubes at 44.5 ± 0.2°C for 24 h ± 2 h.
3. If required, perform the indole test by adding few drops of Kovac's Reagent (REF 19171000) to the EC Broth MUG tubes.

For partitioning of *E. coli* from MF Total Coliform proceed as following[9]:

1. Remove membrane containing total coliform colonies from the medium (e.g. LES Endo Agar) and carefully curl and insert into a tube of EC Broth MUG. Alternatively remove the colonies with a swab and inoculate a tube of EC Broth MUG or, if quantification is required, inoculate individual colonies into the broth.
2. Within 30 minutes immerse all test tubes in a water bath incubator at 44.5 ± 0.2°C for 24 h ± 2 h

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10 - READING AND INTERPRETATION

Presence of growth (turbidity) and a bright blue fluorescence under a long-wave (366 nm) UV light (with or without the production of gas) are considered confirmatory for the presence of *E. coli*.[7]
Indole test: appearance of distinct red colour in upper layer is positive test.

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/24H/A	growth, with fluorescence under Wood's lamp
E. aerogenes ATCC 13048	44°C/24H/A	growth, without fluorescence under Wood's lamp
P. aeruginosa ATCC 27853	44°C/24 H/A	inhibited

A: aerobic incubation; 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 EC Broth MUG is tested for productivity, specificity and selectivity by comparing the results with a previously approved Reference Batch.
Productivity is tested by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of target organisms in test tubes, incubating at 44°C for 24 hours and recording the highest dilution showing growth, gas production and fluorescence under Wood's lamp in Reference Batch (Gr_{RB}) and in Test Batch (Gr_{TB}).
Productivity is tested with the following target strains: *E. coli* ATCC 25925 and *E. coli* ATCC 8739. The productivity index Gr_{RB}-Gr_{TB} for each test strain is ≤ 1 and the tubes exhibit gas into the Durham tubes and fluorescence under Wood's lamp.
Specificity is tested with appropriate dilutions of non-target strains S. Typhimurium ATCC 14028 and *E. aerogenes* ATCC 13048. After incubation the strains exhibits good growth without fluorescence under Wood's lamp.
Selectivity is tested with appropriate dilutions of non-target strains *P. aeruginosa* ATCC 27853 and *E. faecalis* ATCC 19433. After incubation of inoculated tubes, the growth of non-target strains 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 and fluorescent under Wood's Lamp; only exceptionally this test is positive with *Providencia*, *Enterobacter* and *Yersinia* strains (1-5%).[10-12]
- Approximately 3-4% of *E. coli* are β-glucuronidase negative, notably *E. coli* O157 strains[11].
- Up to 10% of *E. coli* have been reported to be slow or non-lactose fermenting but should be MUG-positive.[8,13]
- Since the incubation temperature is critical, the use of submerged waterproofed culture is recommended or the use of an incubator that is documented to hold the temperature at 44.5°C± 0.2°C throughout the chamber over a 24 hours period.[9]

14 - PRECAUTIONS AND WARNINGS

- This product is 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 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.
- Apply Good Manufacturing Practice in the production process of prepared media.
- 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 sterilized plates 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 product 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 and the applied storage conditions (temperature and packaging). According to FDA-BAM, the prepared EC Broth MUG can be stored in the refrigerator for up to 1 month in screw cap tubes.[8]

16 - REFERENCES

1. Hajna AA, Perry CA. Comparative study of presumptive and confirmative media for bacteria of the coliform group and for fecal streptococci. Am J Public Health 1943; 33:550-556.
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12. Kaluzewski S, D Tomczuk D. Evaluation of the Usefulness of Tests for Productionof Beta-D-glucuronidase and Propylene Glycol Utilization for the Differentiation of Enterobacteriaceae Rods. Med Dosw Mikrobiol, 1995; 47:155-68.
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TABLE OF APPLICABLE SYMBOLS

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

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/07

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

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