



EC BROTH

Dehydrated and ready-to-use culture medium

1 - INTENDED USE

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

2 - COMPOSITION - TYPICAL FORMULA *

(AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptone	20.0 g
Lactose	5.0 g
Dipotassium hydrogen phosphate	4.0 g
Potassium dihydrogen phosphate	1.5 g
Sodium chloride	5.0 g
Bile Salts n°3	1.5 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}

EC Broth is recommended:

- by ISO 7251³ for the confirmatory MPN test in the procedure for detection and enumeration of *E. coli* in foodstuffs,
- by FDA-BAM⁴ for the confirmatory MPN test for faecal (thermotolerant) coliforms and *E. coli* in foods, seawater and shellfish,
- by APHA⁵ for the confirmatory test in water samples.

The EC Broth tubes are inoculated with broth from positive presumptive tubes of Lauryl Sulphate Broth and incubated at 44°C (for *E. coli* according to ISO 7252)³ or at 44.5 ± 0.2°C (for thermotolerant coliforms and *E. coli* according to FDA-BAM and APHA)^{4,5}, for 24 ± 2 h. If gas is produced, the test is positive, indicating the presence of thermotolerant coliforms or *E. coli*.

Tryptone provides nitrogen, carbon and minerals for microbial growth; lactose is a fermentable carbohydrate. Phosphates act as buffer system and sodium chloride maintains the osmotic balance. Bile salts n° 3, inhibit the growth of Gram-positive bacteria, especially bacilli and enterococci.

4 - DIRECTIONS FOR MEDIUM PREPARATION

Suspend 37 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	6.8 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
EC Broth	Dehydrated medium	4014252	500 g (13.5 L)
EC Broth	Ready-to-use tubes	551425	20 x 10 mL with Durham tubes

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

Products intended for human consumption and the feeding of animals, and environmental samples in the area of food production and food handling.³ Foods, seawater and shellfish.⁴ For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

9 - TEST PROCEDURE

For the confirmatory test of *E. coli* / faecal coliforms proceed as following:

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.

Incubate the tubes at 44 °C ± 1°C³ (or 44.5 ± 0.2°C)⁴ for 24 h ± 2 h.

If, at this stage, there is no visible gas in the EC broth, extend the incubation up to a total of 48 h ± 2 h. For live shellfish, the total incubation time shall be limited to 24 h ± 2 h.³

10 - READING AND INTERPRETATION

The medium becomes turbid when bacteria are growing; gas formation can be observed as bubbles production accumulated into Durham tubes.

Consider as positive for faecal coliform bacteria the EC Broth tubes with the production of gas.⁴

Consider as positive for *E.coli* bacteria the EC Broth tubes with the production of gas, and which at 44°C produce indole from tryptophan.³



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
<i>E. coli</i> ATCC 25922	44°C/24-48 H/A	growth, with gas production
<i>P. aeruginosa</i> ATCC 27853	44°C/24-48 H/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 Broth are 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 24-48 hours and recording the highest dilution showing growth and gas production 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.

Specificity is tested with appropriate dilutions of non-target strain *S. Typhimurium* ATCC 14028. After incubation, the strain exhibits good growth without gas production.

Selectivity is tested with appropriate dilutions (≥ 10,000 CFU/tube) of non-target strains *P. aeruginosa* ATCC 27853 and *E. faecalis* ATCC 19433. After incubation at 44°C for 48 hours of inoculated tubes, the growth of non-target strains is totally inhibited.

13 - PRECAUTIONS AND WARNINGS

- This culture medium 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.
- Be careful when opening screw cap tubes to prevent injury due to breakage of glass.
- Each tube is for single use only; do not transfer or subdivide the tube content in other containers.
- EC Broth ready-to-use tubes are sterilized by autoclaving.
- 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 Sheets 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.

14 - STORAGE CONDITIONS AND SHELF LIFE

Dehydrated medium

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 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 can be stored in the refrigerator for up to 1 month in screw cap tubes.

Ready to use tubes

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














15 - 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.
2. Perry CA, Hajna AA. Further evaluation of EC medium for the isolation of coliform bacteria and Escherichia coli. Am. J. Public Health 1944; 34:735-738.
3. ISO 7251:2005 Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of presumptive Escherichia coli — Most probable number technique
4. FDA-BAM Chapter 4: Enumeration of Escherichia coli and the Coliform Bacteria. Content current as of:10/09/2020
5. APHA Standard Methods for the Examination of Water and Wastewater, 23rd ed. 2017.





TABLE OF APPLICABLE SYMBOLS

 or  Catalogue number	 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	 For single use only

REVISION HISTORY

Version	Description of changes	Date
Revision 2	Updated layout and content	2022/07

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

