ENTEROBACTERIACEAE (EE) BROTH MOSSEL

Dehydrated culture medium

1 - INTENDED USE

For the detection and enumeration of *Enterobacteriaceae* in foods.

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

Peptone	10.000 g
Glucose	5.000 g
Disodium hydrogen phosphate	6.450 g
Potassium dihydrogen phosphate	2.000 g
Oxgall	20.000 g
Brilliant green	0.014 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

Enterobacteriaceae can be injured in food-processing procedures and are usually considered by food manufacturers as hygiene indicators and thus used to monitor the effectiveness of preventive measures taken.
Enterobacteriaceae Enrichment (EE) Broth Mossel is a modification of brilliant green bile lactose broth designed by Mossel, Visser and Cornelissen[1], which in turn is a modification of MacConkey's liquid medium.
The medium is prepared according to the formulation described in the withdrawn ISO 21528:2004 Standard.[2]
EE Broth Mossel can be used for the selective enrichment, detection and the enumeration with the MPN technique of *Enterobacteriaceae,* mainly when the target microorganisms are expected to be in low number and need resuscitation.
The medium contains brilliant green and bile as the inhibitory agents for Gram-positive bacteria, glucose as the main energy source and peptone which provides the essential factors for growth. Phosphates are the buffering agents to control the pH in the medium and the inhibition of growth in earlier stages of enrichment and auto sterilisation at the end.[3]

4 - DIRECTIONS FOR MEDIUM PREPARATION

Suspend 43.5 g in 1000 mL of cold purified water. Heat to dissolve completely and dispense 100 mL portions in 250 mL flasks (or 10 mL in tubes) and autoclave at 121°C for 5 minutes. Cool rapidly in cold running tap water. If necessary, prepare the medium in double concentration by weighing 87 g/L.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	green, fine, homogeneous, free-flowing powder
Solution and prepared tube appearance	green, limpid
Final pH at 20-25 °C	7.2 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
EE Broth Mossel	Dehydrated medium	4014662	500 g (11.5 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

8 – SPECIMENS

Materials of sanitary importance such as products intended for human consumption and the feeding of animals, environmental samples in the area of food production and food handling. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.

9 - TEST PROCEDURE

General procedure

Take a sample (x g or x mL) depending on the sensitivity required and add 9x mL of Buffered Peptone Water (REF 401278) and homogenise.
Transfer the appropriate volume to a sterile container according to the required detection limit. Incubate at 37 °C for 18 h ± 2 h.
Transfer 1 mL of the culture obtained in Buffered Peptone Water to a tube containing 10 mL of EE Broth Mossel.
Incubate the inoculated broth under aerobic conditions at 37 °C for 22 ± 2 hours.
Using a loop, streak the surface of a dish containing Violet Red Bile Glucose Agar (REF 402188) with the incubated enrichment medium and incubate the dish at 37 °C for 24 h ± 2 h hours.

Enumeration of *Enterobacteriaceae* using the MPN technique

Inoculate single and double concentration EE Broth tubes in triplicate with 1 mL and 10 mL of sample and its decimal dilutions respectively. Incubate at 37°C for 22 ± 2 hours.
Streak a loopful from each tube on Violet Red Bile Glucose Agar plates and incubate at 37°C for 24 h ± 2 hours.

Presence-absence procedure

- Inoculate 1 g or 1 mL of suitably diluted feed into a bottle containing 10 mL Tryptic Soy Broth (REF 402155) and incubate at 20-25°C for 2 hours, shaking the bottle every 15 minutes for 30 seconds.
- Add 10 mL of prepared EE Broth at double concentration and incubate at 37°C for 22 ± 2 hours.
- Mix well and observe for turbidity due to bacterial growth.
- From the growth-positive bottles, transfer a loopful onto a Violet Red Bile Glucose Agar plate and incubate at 37°C for 24 h ± 2 h hours.

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10 - READING AND INTERPRETATION

Turbidity with some change of colour towards yellowish-green provides presumptive evidence of the presence of *Enterobacteriaceae*. Typical *Enterobacteriaceae* colonies on Violet Red Bile Glucose (VRBG) Agar are pink to red or purple (with or without precipitation haloes).

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 some test strains useful for the quality control of the medium.

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
E. coli ATCC 8739	37°/ 24 H-A	growth with gas
E. faecalis ATCC 19433	37°/ 24 H-A	partially 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 EE Broth Mossel is tested for productivity 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 37°C for 24 hours and recording the highest dilution showing growth in Reference Batch (Gr_{RB}) and in Test Batch (Gr_{TB}). Productivity is tested with the following target strains: *E. coli* ATCC 8739, *E. aerogenes* ATCC 13028S, Typhimurium ATCC 14028. The productivity index Gr_{RB}-Gr_{TB} for each test strain shall be ≤ 1.
Productivity and selectivity are tested also together with mixtures of appropriate dilutions of target and non-target strains: *E. coli* ATCC + *E.faecalis* ATCC 19433 and *S.* Enteritidis ATCC 13076+*E. faecalis* ATCC 19433. After incubation of inoculated tubes at 37°C for 24 hours and sub-culture on VRBG Agar, the target strains will show more than 10 colonies on plated medium.
Moreover, selectivity is assessed by dilution to extinction method, by inoculating in test tubes 1 mL of appropriate decimal dilutions of non-target organism *E. faecalis* ATCC 19433, incubating at 37°C for 24 hours and recording the highest dilution showing growth in Reference Batch (Gr_{RB}) and in Test Batch (Gr_{TB}). *E. faecalis* is partially inhibited and the selectivity index Gr_{RB}-Gr_{TB} shall be ≥ -1.

13 – LIMITATIONS OF THE METHOD

- This medium is heat sensitive, avoid overheating.

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 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.
- 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 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 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

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 (tubes/bottles), and the applied storage conditions (temperature and packaging). According to Baird RM *et al.* the tubed broth can be stored in screw-capped containers at +2°C / +8°C for 1 month.[3]

16 – REFERENCES

1. Mossel DAA, Visser M, Cornelissen AMR. The Examination of Foods for Enterobacteriaceae using a Test of the Type Generally Adopted for the Detection of Salmonellae. J Appl Bacteriol 1963; 26:444.
2. ISO 21528-1:2004 - Horizontal methods for the detection and enumeration of Enterobacteriaceae - Part 1: Detection and enumeration by MPN technique with pre-enrichment.
3. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 5:216-217.

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 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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