ENTEROBACTERIA ENRICHMENT BROTH MOSSEL EP

Dehydrated culture medium

1 - INTENDED USE

For the detection and enumeration of bile-tolerant Gram-negative bacteria in pharmaceutical products.

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

Pancreatic digest of gelatin	10.000 g
Glucose anhydrous	4.500 g *
Bile Salts	3.055 g **
Potassium dihydrogen phosphate	2.000 g
Disodium hydrogen phosphate anhydrous	6.400 g ***
Brilliant green	0.015 g

^The formula may be adjusted and/or supplemented to meet the required performances criteria.
* Equivalent to 5.0 g di glucose monohydrate
** Equivalent to 20 g di dehydrated ox bile
*** Equivalent to 8 g di disodium hydrogen phosphate dihydrate

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Enterobacteria Enrichment Broth Mossel EP 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. Enterobacteria Enrichment Broth Mossel EP is recommended as an enrichment medium for bile-tolerant Gram-negative bacteria in the microbiological examination of pharmaceutical products. It can be used as test for absence or quantitative test.[2] The medium contains brilliant green and bile as the inhibitory agents for Gram-positive bacteria, glucose as the main energy source and pancreatic digest of gelatin 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 26 g in 1000 mL of cold purified water. Mix thoroughly to completely dissolve the powder. Dispense 100 mL portions in 250 mL flasks (or 10 mL in tubes). Heat the medium at 100°C using free flowing steam for 30 minutes only. Cool rapidly in cold running tap water. Do not autoclave.

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
Enterobacteria Enrichment Broth Mossel EP	Dehydrated medium	4014672	500 g (19 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

8 – SPECIMENS

Non-sterile pharmaceutical products. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.[2]

9 - TEST PROCEDURE

Prepare the sample suspension in Tryptic Soy Broth (REF 402155) using at least 1 g or 1 mL of sample.
Incubate this suspension at 20°C - 25°C for 2-5 hours to ensure revitalisation but not multiplication of bacteria.

a- Test for absence

Inoculate a quantity of the initial suspension into Enterobacteria Enrichment Broth Mossel EP to ensure an inoculum of 1 g of sample and incubate at 30°C - 35°C for 24-48 hours
Subculture on plates of VRBG Agar EP (REF 402189) and incubate 30°C -35°C for 18-24 hours.

b- Quantitative test

Inoculate suitable quantities of Enterobacteria Enrichment Broth Mossel EP with the initial suspension and/or dilution of sample containing respectively 1 g, 0.1 g, 0.01 g and 0.001 g of the product to be examined. Incubate at 30°C - 35°C for 24-48 hours.
Subculture each of the cultures on a plate of VRBG Agar EP (REF 402189) and incubate 30°C -35°C for 18-24 hours

10 - READING AND INTERPRETATION

Turbidity in the medium with some change of colour towards yellowish-green provides presumptive evidence of the presence of bile-tolerant Gram-negative bacteria.
Typical glucose fermenters colonies on VRBG Agar EP are pink to red or purple (with or without precipitation haloes).
Typical non-glucose fermenters colonies on VRBG Agar EP are transparent and colourless.
Test for absence: according to European Pharmacopoeia the product complies with the test if there is no growth of colonies on VRBG Agar EP plates.
Quantitative test: refer to European Pharmacopoeia for the interpretation criteria of probable number of bacteria per g or mL of product.

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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	30-35°/ 24 H-A	growth
P. aeruginosa ATCC 9027	30-35°/ 24 H-A	growth
S. aureus ATCC 6538	30-35°/ 48 H-A	growth 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 Enterobacteria Enrichment Broth Mossel EP is tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.
Productivity is tested by inoculation less than 100 CFU of target organisms *E. coli* ATCC 8739 and *P. aeruginosa* ATCC 9027 per test tube and incubating at 30-35°C for 24 hours. The target strains exhibit good growth.
Selectivity is tested by inoculation less than 100 CFU of non-target organisms *S. aureus* ATCC 6538 and incubating at 30-35°C for 48 hours. *S. aureus* is totally inhibited.

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 tubes/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

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, the self-prepared tubes or bottles can be stored at +2°C +8°C in in screw-capped containers for up to four weeks.[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. European Pharmacopoeia 11th Edition, 2022, Vol. 1; 2.6.13 Microbiological Examination of non-sterile products: test for specified micro-organisms: 01/2021:20631
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 1	Updated layout and content	2022/11

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

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