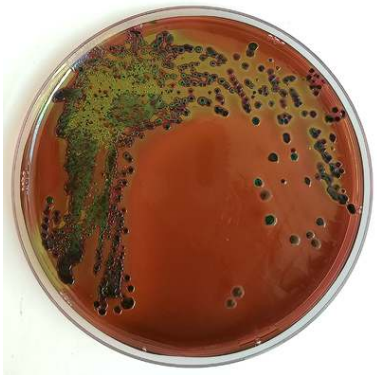
EO. ME. BLUE AGAR WITH LACTOSE AND SUCROSE

Dehydrated culture medium



Eo.Me.Blue Agar with Lactose and Sucrose: colonies of *E.coli*

1 - INTENDED USE

For the isolation of *Enterobacteriaceae* and for the differentiation of lactose/sucrose-fermenting microorganisms.

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

Tryptone	10.000 g
Sodium chloride	5.000 g
Lactose	5.000 g
Sucrose	5.000 g
Dipotassium hydrogen phosphate	2.000 g
Eosin yellow	0.400 g
Methylene blue	0.065 g
Agar	15.000 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

Eo.Me.Blue Agar with Lactose and Sucrose is prepared on the basis of the formulation described by Holt-Harris & Teague in 1916.[1]
Compared to Levine's modified formula[2], this medium contains sucrose in addition to lactose which is fermented by certain enteric bacteria more readily than lactose.
Eo.Me.Blue Agar with Lactose and Sucrose is a versatile, moderately selective medium for the isolation and differentiation of *Enterobacteriaceae* based on the fermentation of lactose and sucrose, from a variety of specimens.
The simultaneous presence of lactose and sucrose allows to differentiate lactose and sucrose-negative pathogens from lactose positive coliforms and lactose-negative, sucrose-positive flora (e.g. *Proteus vulgaris, Citrobacter, Aeromonas hydrophila*)
Peptone provides nitrogen, carbon, minerals for microbial growth; eosin yellow and methylene blue have a slight inhibitory activity towards Gram-positive microorganisms; the optimal ratio between the contents of the two dyes is required for the differentiation of lactose/sucrose-fermenting enteric bacteria from non-lactose/sucrose fermenters.
The phosphate buffer allows the differentiation between *E. coli* and *E. aerogenes*. *E. coli* causes considerable acidification of the medium even in the presence of a buffer system, whereas *E. aerogenes*, being only slightly fermenting, causes less acidification. The lowering of pH during *E. coli* growth, causes the formation of amidic bonds between the eosin and the methylene blue, which manifests as a metallic purple coloration of the colonies.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 42.5 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to about 50°C and before distributing to plates, gently shake the flask to disperse the flocculent precipitate that is formed during sterilisation. Flocculent precipitate should not be removed.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	violet, fine, homogeneous, free-flowing powder
Solution appearance	violet with metallic sheen, flocculent, hazy
Prepared plates appearance	violet, limpid or slightly hazy
Final pH at 20-25 °C	7.2 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Eo.Me.Blue Agar with Lactose and Sucrose	Dehydrated medium	40145012	500 g (11.7 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and swabs, incubator and laboratory equipment as required, Petri dishes, Erlenmeyer flasks, ancillary culture media and reagents for the identification of the colonies.

8 - SPECIMENS

Eo.Me.Blue Agar with Lactose and Sucrose is intended for the bacteriological processing a variety of specimens on which detect *Enterobacteriaceae*. Good laboratory practices for collection, transport and storage of the specimens should be applied.

9 - TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.
Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
Incubate in aerobic conditions at 35-37°C for 18-24 hours.

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
E. coli colonies are 2-3 mm in diameter, slightly raised, concave, rarely convex; they are violet-cyclamen with a darker centre that extends for about 3/4 of the diameter, with greenish metallic sheen.

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E. aerogenes colonies are convex with a diameter of about 4-6 mm, pink to lavender in colour, with a darker centre smaller than that observed with *E. coli*; they are normally free of greenish metallic sheen.
The colonies of sucrose fermenters such as *Proteus* spp. are violet colonies and the medium may exhibit metallic sheen.
The colonies of non-lactose/sucrose fermenters (*Salmonella, Shigella*) are transparent, amber or pink or colourless.

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	35-37°C / 18-24 H / A	growth, violet-cyclamen colonies with a darker centre with metallic sheen
E. aerogenes ATCC 13048	35-37°C / 18-24 H / A	growth, dark pink colonies
S. Typhimurium ATCC 14028	35-37°C / 18-24 H / A	growth, colourless or whitish colonies
E. faecalis ATCC 19433	35-37°C / 18-24 H / A	growth 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 Eo.Me.Blue Agar with Lactose and Sucrose are tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.
Productivity is tested by semi-quantitative ecometric technique, by incubating at 37°C for 24 hours, with the following Gram-negative strains: *E. coli* ATCC 25922, *E. coli* ATCC 8739, *E. aerogenes* ATCC 13048, *K. pneumoniae* ATCC 27736, *C. freundii* ATCC 8090, S. Typhimurium ATCC 14028, *S. flexneri* ATCC 12022, *P. vulgaris* ATCC 9484, *P. mirabilis* ATCC 10005. After incubation the colours and characteristics of the colonies and the amount of growth are evaluated and recorded. All strains grow with typical colonies and the growth is comparable in both batches.
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 strains *E. faecalis* ATCC 19433 and *S. aureus* ATCC 25923. Non-target strains are partially inhibited and grow with colourless, pinpoint colonies.

13 - LIMITATIONS OF THE METHOD

- Eo.Me.Blue Agar with Lactose and Sucrose is only moderately selective; some staphylococci, streptococci and yeasts grow exhibiting small, pinpoint colonies.[3]
- Some strains of *Salmonella* and *Shigella* will not grow on the medium.[3]
- Store prepared medium in the dark at 2-8°C; the photosensitive dyes in the medium may inhibit growth of certain bacteria, mainly *Proteus*, if stored in light.[4]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, perform the suitable tests on isolates from pure culture for complete identification.

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.
- Apply Good Manufacturing Practice in the production process of prepared media.
- 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.
- 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 media 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 for the validation of the shelf life of the finished products, according to the type (plates/tubes/bottles) and the storage method (temperature and packaging). According to MacFaddin the self-prepared plates can be stored in the dark at 2-8°C for 6-8 weeks.[3]

16 - REFERENCES

1. Holt-Harris JE, Teague O. A new culture medium for the isolation of Bacillus typhosus from stools. J Inf Dis 1916; 18:596-600
2. Levine M. Differentiation of B coli and B aerogenes on a simplified eosin-methylene blue agar J Inf Dis 1918; 23:43-47
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
4. Girolami RL Stamm JM (1976) Inhibitory Effect of Light on Growth-Supporting Properties of Eosin Methylene Blue Agar. Appl Environ Microbiol 1976;31: 141–142

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	2022/08

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