INSTRUCTIONS FOR USE

LEVINE EMB BLUE AGAR

Ready-to-use plates

Levine EMB Agar: colonies of *E.coli* with greenish metallic sheen and S.Typhimurium (pinkish colonies)

1 - INTENDED USE

In vitro diagnostic device. Medium for the isolation and differentiation of *Enterobacteriaceae* from clinical and non-clinical specimens.

2 - COMPOSITION -TYPICAL FORMULA *

Peptone	10.000 g
Lactose	10.000 g
Dipotassium hydrogen phosphate	3.000 g
Eosin yellow	0.400 g
Methylene blue	0.065 g
Agar	14.000 g
Purified water	1000 mL

*the formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Levine EMB Blue Agar has been formulated by Levine in 1918[1], as a modification of the Holt-Harris & Teague EMB (HHT) medium devised in 1916[2]. Compared to HHT, Levine EMB Blue Agar contains a single sugar, lactose, at higher concentration and, according to Levine[1], this modification allows a better differentiation between the species that are now called *Escherichia coli* and *Enterobacter aerogenes*.

Levine EMB Blue Agar is a versatile, moderately selective medium for the isolation and differentiation of *Enterobacteriaceae* based on the fermentation of lactose, from clinical specimens[3,4] and other materials. Its use for cosmetics,[5] food,[6] dairy products,[7] water[8] and pharmaceutical products[9] has been described.

The peptone provides nitrogen, carbon, minerals for microbial growth; lactose is included as a fermentable carbohydrate. The dye methylene blue partially inhibits the growth of Gram-positive bacteria. Eosin yellow is a dye that responds to changes in pH, going from colourless to black under acidic conditions. Lactose-fermenting gram-negative bacteria (generally enteric) acidify the medium, and under acidic conditions the dyes produce a dark purple complex which is usually associated with a green metallic sheen.[10] The differentiation between *E.coli* and *E.aerogenes* is made possible by the presence of the phosphate buffer which minimizes the acidifying effects produced by the slow fermentation of lactose by *E.aerogenes*.

4 - PHYSICAL CHARACTERISTICS

Medium appearance	green-violet, limpid or slightly hazy
Final pH at 20-25°C	7.1 ± 0.2

5 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Levine EMB Blue Agar	Ready-to-use plates	541595	2 x 10 plates ø 90 mm primary packaging: 2 cellophane sachets secondary packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops and swabs, incubator and laboratory equipment as required, ancillary culture media and reagents for the identification of the colonies.

7 - SPECIMENS

Levine EMB Blue Agar is intended for the bacteriological processing of a variety of clinical specimens on which detect *Enterobacteriaceae*. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, storage and transport of the specimens to the Laboratory should be applied. For non-clinical samples, refer to the applicable international Standards.

8 - 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.

9 - 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.

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 lactose non-fermenting organisms (*Salmonella, Shigella, Proteus* etc.) are transparent, amber, pink or colourless.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: mktg@biolifeitaliana.it; export@biolifeitaliana.it; web: www.biolifeitaliana.it

10 - 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.[11]

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 and a metallic sheen
S.Typhimurium ATCC 14028	35-37°C / 18-24 H / A	growth, colourless to amber 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

11 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of ready to use plates of Levine EMB Blue Agar and of the raw material used for the production of prepared plates (dehydrated Levine EMB Blue Agar REF 401595) 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 35-37°C for 18-24 hours, with the following Gram-negative strains: *E.coli* ATCC 25922, *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 is evaluated and recorded. All strains grow well with typical colonies.
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 29212, *E.faecalis* ATCC 19433 and *S.aureus* ATCC 25923. Non-target strains are partially inhibited and grow with colourless, pinpoint colonies.

12 - LIMITATIONS OF THE METHOD

- Levine EMB Blue Agar is only moderately selective; some staphylococci, streptococci and yeasts grow exhibiting small, pinpoint colonies. As well, other Gram-negative non-fermenting bacilli exhibit growth appearing as non-lactose fermenters (e.g. *Aeromonas*, *Acinetobacter* and *Pseudomonas*).[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.[12]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification. If relevant, perform antimicrobial susceptibility testing.
- This culture medium is intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

13 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This product is not classified as dangerous according to current European legislation.
- 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 ready-to-use plates 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.
- Each plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- 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.
- 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.

14 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g., microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

15 - REFERENCES

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7. Standard Methods for the Examination of Dairy Products, 13th Ed. APHA, 1972
8. Standard Methods for the Examination of Water and Wastewater, 14th Ed APHA, 1975
9. United States Pharmacopoeia XXI (1985) Microbial. Limit Tests. Rockville. Md.
10. Archana Lal, Naowarat Cheeptham. Eosin-Methylene Blue Agar Plates Protocol. American Society for Microbiology, 2011
11. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004.
12. Girolami RL, Stamm JM. 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	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	For single use only	Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 2	Updated layout and content in compliance with IVDR 2017/746	2021/01
Revision 3	Removal of obsolete classification	2023/03

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

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