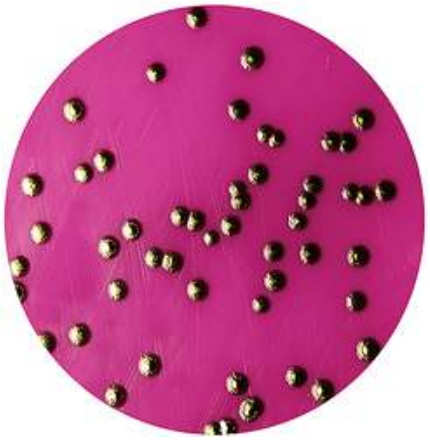
m-LES ENDO AGAR

Dehydrated and ready-to-use culture medium



m-LES Endo Agar: *E.coli* colonies

1 - INTENDED USE

For the enumeration of coliforms in water samples by membrane filtration.

2 – COMPOSITION*

TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Yeast extract	1.20 g
Tryptone	3.70 g
Peptone	3.70 g
Tryptose	7.50 g
Lactose	9.40 g
Dipotassium hydrogen phosphate	3.30 g
Potassium dihydrogen phosphate	1.00 g
Sodium chloride	3.70 g
Sodium deoxycholate	0.10 g
Sodium lauryl sulphate	0.05 g
Sodium sulphite	1.60 g
Basic fuchsin	0.80 g
Agar	15.00 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

Endo Agar was originally developed by Endo[1] for the isolation of the typhoid bacillus. McCarthy, Delaney, and Grasso[2] modified Endo's formulation and proposed the LES (Lawrence Experimental Station) Endo Agar, for the recovery of coliforms with a membrane filter 2 steps technique: 1- pre-enrichment of the filter in Lauryl Sulphate Broth, 2- incubation of the filter on a LES Endo Agar plate. McCarthey *et al.* recovered higher numbers of coliforms with the 2 steps method compared to the one-step technique with m-Endo medium.[2]
Both one and two steps Membrane Filter procedures have been included in the APHA Standard Methods for the detection of coliforms in drinking, non-potable, and other waters.[3]
In m-LES Endo Agar, essential growth factors are provided by peptones which are sources of nitrogen, carbon and minerals. Yeast extract is a source of vitamins, particularly of the B-group. Phosphates are used as buffering agents to control the pH in the medium. Sodium chloride is a source of electrolytes and maintains the osmotic equilibrium. The slight inhibition of Gram-positive bacteria achieved with the sodium sulphite/acid fuchsin combination in classical Endo formulation, has been improved in "LES" formulation by inclusion of sodium deoxycholate and sodium lauryl sulphate. The sodium sulphite in the medium also has the function of decolourising acid fuchsin as it does in Schiff's reagent. Lactose-fermenting bacteria produce acetaldehyde from lactose which releases the fuchsin from the colourless fuchsin-sulphite compound and colours the colonies red; when the reaction is rapid and very intense (e.g in the case of *E. coli*), the fuchsin crystallises and produces a metallic sheen on the colonies. In areas of the plate with intense growth, the metal sheen is suppressed. Non-lactose-fermenting organisms produce colourless colonies against the pink background of the medium.

4 - DIRECTIONS FOR DEHYDRATED MEDIUM

Suspend 51 g in 980 mL of cold purified water and add 20 mL of 95% ethanol. Heat to boiling with frequent agitation to dissolve completely. Cool to 47-50°C, mix well for resuspending the precipitate and distribute into sterile Petri dishes. Do not autoclave, avoid direct sunlight.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	purple, fine, homogeneous, free-flowing powder with small dark particles
Solution appearance	pink-red, slightly opalescent to opalescent with precipitate
Prepared medium appearance	pink-red, slightly opalescent
Final pH at 20-25 °C	7.1 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
m- LES Endo Agar	Dehydrated medium	4015932	500 g (9.8 L)
LES Endo Agar	Ready-to-use plates	541593	2 x 10 plates ø 90 mm
LES Endo Agar	Ready-to-use plates	491543	3 x 10 plates ø 55 mm

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, sterile Petri dishes, 95% ethanol, membrane filtration system, ancillary culture media and reagents.

8 – SPECIMENS

Water samples. Consult the appropriate references for sample collection, storage and preparation.[3,4]

9 - TEST PROCEDURE

One step technique.[3]

1. Using an appropriate sterile filtration unit, filter the water sample.
2. Aseptically, place the membrane filter on the m-LES Endo Agar plate, invert dish and incubate for 22 to 24 hours at 35°C

Two steps technique.[3]

1. Place an absorbent pad in a 55 mm Petri dish and pipette at least 2 mL of Lauryl Pepto Bios Broth (REF 401580), to saturate pad.
2. Using an appropriate sterile filtration unit, filter the water sample.
3. Aseptically, place the membrane filter on the pad and incubate for 1.5 - 2 hours at 35°C in a moist atmosphere.

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

4. Transfer the membrane from the pad to 55 mm Petri dish containing m-LES Endo Agar, avoiding the formation of air bubbles between the filter and the agar surface.
5. Incubate at 35°C for 20 to 24 hours.

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Typical coliforms colonies are pink to red with metallic sheen. The sheen may cover the entire colony or may only appear in the centre or on the periphery.
Some colonies will appear pink or red but lack the characteristic metallic sheen. These colonies are classified as atypical coliforms and need to be verified through further testing.
Typical non-lactose fermenters colonies are colourless against the pink-red background of the medium

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	37°C/24H-A	good growth, pink-red colonies with metallic sheen
S. Enteritidis ATCC 13076	37°C/24H-A	good growth, colourless colonies

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12 – PERFORMANCE CHARACTERISTICS

Prior to release for sale, representative samples of all lots of dehydrated and ready-to-use m-LES Endo Agar (Test Batch TB) are tested for productivity, specificity and selectivity by comparing the results with a previously approved Reference Batch (RB).
The productivity is tested by a quantitative method with the target strains *E. coli* ATCC 25922 and *E. aerogenes* ATCC 13048: the membrane filter on the plate is inoculated with decimal dilutions in saline of a colonies' suspension and incubated at 37°C for 20-24 hours. The colonies are enumerated on both batches and the productivity ratio (Pr:UFC_{TB}/UFC_{RB}) is calculated. If Pr is ≥ 0.7 and if the colonies morphology and colour are typical (pink-red colonies with metallic sheen) the results are considered acceptable and conform to the specifications.
Moreover, the productivity and specificity characteristics are tested by semi-quantitative ecometric technique with the following strains: *E. coli* ATCC 8739, *K. pneumoniae* ATCC 23357 and S. Enteritidis ATCC 13076. After incubation, the amount of growth and the colony characteristics are evaluated: coliforms strains exhibit good growth with pink-red colonies with metallic sheen whereas S. Enteritidis grows with colourless colonies.
The selectivity is assessed with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of *S. aureus* ATCC 25923 and *E. faecalis* ATCC 19433. The growth of Gram-positive strains is totally inhibited.

13-LIMITATIONS OF THE METHODS

- Occasionally, non-coliform organisms may produce typical sheen colonies.
- Occasionally, some colonies will appear pink or red but lack the characteristic metallic sheen. These colonies are classified as atypical coliforms and need to be verified with further tests.

14 - 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 m-LES Endo Agar is classified as dangerous since contains acid fuchsin, a potential carcinogen. 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.
- Each ready-to-use 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.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder 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 Sheets of the products 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

Ready to use plates

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 in the dark. 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).

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 (plates/tubes/bottles) and the applied storage conditions (temperature and packaging). According to APHA, the self-prepared plates may be stored at 2-8°C in the dark, preferably in sealed plastic bag, for up to 2 weeks.[3] Discard the medium sooner than 2 weeks if there is evidence of moisture loss, medium contamination, medium darkening, or surface sheening formation.[3]

16 - REFERENCES

1. Endo S. Über ein Verfahren zum Nachweis der Typhus bacillen. Centr f Bakt 1904; 35:109-110.
2. McCarthy JA, Delaney JE, Grasso RJ. Measuring coliforms in water. Water Sewage Works 1961; 108:238
3. APHA. Standard methods for the examination of water and wastewater, 23th ed., 2017. American Public Health Association, Washington, D.C.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT 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 1	Updated layout and content	2022/08

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

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