



EMB Levine Agar

Selective medium for detection and isolation of Enterobacteriaceae.

INTENDED PURPOSE

Selective medium for isolation of enteric Gram-negative bacteria from clinical specimens. This medium is intended as an aid in diagnosis, requiring further tests to complete the diagnostic results.

DESCRIPTION

Eosin Methylene Blue (EMB) Levine Agar allows to distinguish enteric pathogens from accompanying flora on the basis of the ability of bacteria to ferment the sugars in the medium.

TYPICAL FORMULA*

	(g/litre)
Peptone	10.0
Lactose	10.0
Potassium phosphate bibasic	2.0
Eosine Y	0.4
Methylene blue	0.066
Agar	15.0
Final pH 7.1 ± 0.2	

*Adjusted and/or supplemented as required to meet performance specifications.

METHOD PRINCIPLE

Peptone is the nitrogen source, lactose is the fermentable carbohydrate, and dipotassium phosphate is the buffer. Eosin Y and methylene blue are the indicators. These dyes also play a role in differentiating between lactose fermenters and lactose non fermenters due to the presence or absence of dye uptake in the bacterial colonies. Methylene blue works also as selective agent inhibiting gram- positive bacteria to a limited degree.

PREPARATION

Dehydrated medium.

Suspend 37.5 g of powder in 1 liter of distilled or deionized water. Mix well. Heat until dissolved completely. Sterilize in autoclave at 121°C for 15 minutes. Cool at 45-50°C, mix well avoiding foam formation and aseptically distribute into Petri dishes.

Medium in bottles

Melt the content of the bottle in a boiling water-bath at 100°C (loosing the caps partially unscrewed) until completely dissolved. Cool to 45-50°C, mix well avoiding the formation of bubbles and aseptically distribute into Petri dishes. Allow the medium to solidify. Store the plates in tightly closed containers.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as: autoclave, sterile Petri plates, test tubes, inoculating loops, swabs, incubator, quality control organisms.

SPECIMENS

Clinical specimens should be sampled at the acute stage, before antimicrobial therapy (where possible) and examined as soon as possible after collection. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied. Refer to specific guidelines for more information about specimen collection and preparation.

TEST PROCEDURE

Inoculate a plate with the sample and streak for isolation.

Incubate plates, protected from light, at 35 ± 2°C for 18-24 hours. If negative after 24 hours, reincubate an additional 24 hours.

INTERPRETING RESULTS

At the end of the incubation period evaluate colonies characteristics as follows:

Lactose-fermenting microorganisms, such as coliforms, are visualized as blue-black colonies, whereas lactose non fermenters, such as *Salmonella* spp and *Shigella* spp, appear colorless, transparent or amber. Some gram-positive bacteria, such as fecal streptococci, staphylococci and yeast, will grow in this medium and usually form pinpoint colonies. A number of non-pathogenic lactose non fermenting gram-negative bacteria will grow in this medium and must be distinguished from pathogenic strains by additional biochemical tests.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

Store bottles and prepared plates at 10-25°C away from light, until the expiry date on the label or until signs of deterioration or contamination are evident.

SHELF LIFE

Dehydrated medium: 4 years.

Ready-to-use plates: 6 months

Medium in bottles: 2 years.

QUALITY CONTROL

Appearance of Dehydrated Medium: Fine, homogeneous, may contain dark red purple particles, reddish.

Appearance of Prepared Medium: Slightly opalescent, Green orange brown.

Expected Cultural Response:

Control strain		Inoculum	Incubation	Criteria	Specification
<i>Escherichia coli</i>	ATCC® 25922	10-100 CFU	18-24 h / 35 ± 2°C	Good growth (P _R ≥ 0.5)	Blue-black colonies with green metallic sheen
<i>Salmonella</i> Typhimurium	ATCC® 14028				Colourless to amber
<i>Shigella flexneri</i>	ATCC® 12022				Colourless to amber
<i>Enterococcus faecalis</i>	ATCC® 29212	10 ⁴ -10 ⁶ CFU	18-24 h / 35 ± 2°C	Partial to complete inhibition	---

A productivity ratio (P_R) of 0.5 is equivalent to a recovery rate of 50%.

Please refer to the actual batch related Certificate of Analysis (CoA).

PERFORMANCE CHARACTERISTICS

Performance testing of EMB Levine Agar was carried out using the QC strains listed above. The results obtained met the established criteria.

LIMITATIONS

Invalid results can be caused by poor specimen quality, improper sample collection, improper transportation, improper laboratory processing, or a limitation of the testing technology. The operator should understand the principles of the procedures, including its performance limitations, in advance of operation to avoid potential mistakes.

Due to nutritional variation, some strains may result in poor growth or fail to grow on this medium.

As the medium is moderately inhibitory some staphylococci, enterococci and yeast may grow. Also some Gram-negative non-fermenting bacilli may appear as non-lactose fermenters.

Some strains of *E. coli* may fail to produce a characteristic green metallic sheen; For that reason, the green metallic sheen is not diagnostic for *E. coli*.

WARNING AND PRECAUTIONS

- 1) For in vitro diagnostic use (IVD).
- 2) For laboratory professional use only.

- 3) Operators must be trained and have certain experience. Please read the instructions carefully before using the product. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this document.
- 4) Consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.
- 5) Do not use if the product or packaging appears to be damaged.
- 6) Follow standard precautions. All patient specimens should be considered potentially infectious and handled accordingly.
- 7) Handle all specimens as if infectious using safe laboratory procedures. Dispose of hazardous or biologically contaminated materials according to the practices of your institution.
- 8) Avoid cross-contamination of samples by using disposable tips and changing them after each sample.
- 9) Do not mix reagents of different batches. Please use the product within the validity period.
- 10) Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- 11) Results should be interpreted by a trained professional in conjunction with the patient's history and clinical signs and symptoms, and epidemiological risk factors.
- 12) Ensure laboratory equipment is calibrated and maintained in accordance with the laboratory's procedure.
- 13) When test results are transmitted from the laboratory to an informatics centre, attention has to be done to avoid erroneous data transfer.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

See the references at the end of this document.

TABLE OF SYMBOLS

See the table of symbols at the end of this document.

ORDER INFORMATION

Product	Format	Packaging	Ref.
EMB Levine Agar	Dehydrated media	500 g	610019
		100 g	620019
	Bottles	6 x 100 ml	402350
	Plates	2 x 10 plates	10048

There may be additional product ref. numbers as well. For an updated listing of available products, visit liofilchem.com

Revision History

Revision	Release Date	Change Summary
0	2025-08-29	Updated layout and content in compliance with IVDR 2017/746, version reset to revision 0

In case of malfunctions or defects, contact immediately Liofilchem (*) or the local representative.

In case of incident associated with the device, notify immediately Liofilchem (*) or its local representative and the National Competent Authority.

*Please login to <https://www.liofilchemstore.it/login.php> (user ID and password required) and click on Complaint.

This IFU document and the SDS are available from the online Support Center:

liofilchem.com/ifu-sds