62087 Levine EMB Agar (Eosin methylene Blue Lactose Agar according to Levine; Levine's Eosin Methylene Blue Agar)

Isolation and differentiation of *E. coli* and *Enterobacter*, and identification of *Candida albicans* acc. to Levine. Used for testing food, water and clinical (urine) samples.

Composition:

Ingredients	Grams/Litre
Meat peptone	10.0
Lactose	10.0
Dipotassium hydrogen phosphate	2.0
Eosin yellowish	0.4
Methylene blue	0.65
Agar	15.0

Final pH 7.2 +/- 0.2 at 25°C

Store prepared media below 8°C and protected from light to avoid photooxidation. Store dehydrated powder in a dry place, in tightly-sealed containers at 2-25°C.

Appearance:	Light purple to purple and pink, homogeneous powder.
Gelling:	Firm
Color and Clarity:	red to dark red and purple to dark purple, clear to slightly turbid, some precipitate possible.

Directions:
Dissolve 37.5 g in 1 litre distilled water. Sterilize by autoclaving at 121°C for 15 minutes. Cool to 60°C and shake the medium in order to oxidize the methylene blue (i.e. restore its blue color) and to suspend the precipitate, which is an essential part of the medium.
Pour plates.

Principle and Interpretation:
Levine described this classical method to identify *E. coli* from other coliforms as *Klebsiella aerogenes* and also for the rapid identification of *Candida albicans* (2, 3). This medium is recommended for the detection, enumeration and differentiation of members of the coliform group by American Public Health Association (9, 14, 15). Weld (4,5) proposed the use of Levine EMB Agar, with added chlortetracycline hydrochloride, for the rapid identification of *Candida albicans* in clinical specimens. A positive identification of *Candida albicans* can be made after 24-48 hours incubation at 35-37°C in 10% carbon dioxide atmosphere, from specimens such as faeces, oral and vaginal secretions and nail or skin scraping etc. However, the typical appearance is variable.
The presence of the colorants eosine yellowish and methylene blue inhibits the growth of most of the common accompanying Gram-positive microorganisms. Lactose is added as distinctive carbon and energy source. In combination with the added dyes it allows to distinguish between lactose-positive and lactose-negative organisms. Lactose positive cultures are generally dark violet to black (coliforms like *Enterobacter, Klebsiella, E. coli*) due to uptake of methylene blue-eosin dye complex, when the pH drops. Lactose negative organisms (*Salmonella, Shigella*) have only peptone as energy source, which results in raise of the pH of surrounding medium, due to oxidative de-amination of protein. This leads to solubilization of the methylene blue-eosin complex and colorless colonies. Peptone serves as source

of carbon, nitrogen, peptides, vitamins and other essential growth nutrients. Dipotassium hydrogen phosphate act as the buffering agent.
The samples can be directly streaked on the medium plates. Inoculated plates should be incubated, protected from light.
Some gram-positive bacteria, such as fecal streptococci, staphylococci may grow on this medium as inhibited small colonies. A number of non-pathogenic, lactose-nonfermenting gram-negative bacteria will grow on this medium and must be distinguished from the pathogenic strains by additional biochemical tests.

Cultural characteristics after 24-48 hours at 35-37°C.

Organisms (ATCC/WDCM)	Inoculum (CFU)	Growth	Recovery	Appearance of Colony
Escherichia coli (NCTC 9002)	50-100	+++	≥50%	Blue-black with green metallic sheen
Escherichia coli (8739/000012)	50-100	+++	≥50%	"
Escherichia coli (25922/00013)	50-100	+++	≥50%	"
Klebsiella aerogenes (13048/00175)	50-100	++	40-50%	Pink-red
Staphylococcus aureus subsp. aureus (6538/00032)	50-100	-/+	≤10%	colorless
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Salmonella Typhimurium (14028/00031)	50-100	+++	≥50%	colorless
Enterococcus faecalis (29212/00087)	50-100	-/+	≤10%	colorless
Pseudomonas aeruginosa (27853/00025)	50-100	+++	≥50%	colorless
Pseudomonas aeruginosa (9027/00026)	50-100	+++	≥50%	colorless
Candida albicans (10231/00054)*	50-100	+++	≥50%	colorless
Saccharomyces cerevisiae (9763/-)	50-100	-/+	≤10%	cream

* key: incubated in 10% carbodioxide

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Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.