# EMB (LEVINE) AGAR

## INTENDED USE
Remel EMB (Levine) Agar is a solid medium recommended for use in qualitative procedures for selective and differential isolation of gram-negative enteric bacilli from clinical specimens.

## SUMMARY AND EXPLANATION
Eosin Methylene Blue Agar (EMB Agar) was originally developed by Holt-Harris and Teague.[1] The combination of dyes in the medium and the incorporation of lactose and sucrose provided a differential plating medium for differentiating enteric gram-negative bacilli. The ratios of eosin and methylene blue in the formula were balanced to give maximum differentiation between organisms which ferment lactose and sucrose and those which do not.[2] The Levine modification of EMB Agar does not contain sucrose, and the lactose concentration is increased.[3] Levine reported this formulation provided better differentiation between *Escherichia coli* and *Enterobacter* species.

## PRINCIPLE
Eosin and methylene blue dyes provide for differentiation of lactose fermenters and nonlactose fermenters based on the uptake of dyes by colonies. Eosin dye combines with methylene blue indicator to produce a color change when lactose is fermented. Coliforms, such as *E. coli*, form blue-black colonies with a green metallic sheen due to the amide bonding of the dyes in an acid condition. Other coliforms, such as *Enterobacter* spp., form mucoid, pink-brown colonies in a less acidic condition. Nonlactose-fermenters, such as *Shigella* and *Salmonella*, are distinguished from coliforms by the formation of transparent, colorless, or amber colonies. Eosin and methylene blue dyes are also selective agents which inhibit gram-positive organisms. The Levine modification does not contain sucrose and therefore, enteric gram-negative bacilli which ferment lactose slowly (e.g., *Citrobacter)* may mimic the appearance of enteric pathogens.

## REAGENTS (CLASSICAL FORMULA)*

| | | | |
|---|---|---|---|
| Gelatin Peptone | 10.0 g | Eosin Y | 0.4 g |
| Lactose | 10.0 g | Methylene Blue | 65.0mg |
| Dipotassium Phosphate | 2.0 g | Agar | 15.0 g |
| | | Demineralized Water | 1000.0 ml |

pH 7.1 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

## PRECAUTIONS
This product is For Laboratory Use only. It is not intended for use in the diagnosis of disease or other conditions.

## PREPARATION OF DEHYDRATED CULTURE MEDIUM
1. Suspend 37.5 g of medium in 1000 ml of demineralized water.
2. Heat to boiling with agitation to completely dissolve.
3. Sterilize by autoclaving at 121°C for 15 minutes or following established laboratory procedures.
4. Dispense into appropriate containers.

## PROCEDURE
1. Consult current editions of appropriate references for the recommended procedure for sample preparation, inoculation, testing, and interpretation.

## QUALITY CONTROL
Each lot number of EMB Levine Agar has been manufactured, packaged, and processed in accordance with current Good Manufacturing Practice regulations. All lot numbers have been tested using the following quality control organisms and found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, sample results should not be reported.

| CONTROL | INCUBATION | RESULTS |
|---|---|---|
| *Escherichia coli* ATCC® 25922 | Aerobic, 18-24 h @ 33-37°C | Growth, blue-black colonies w/ green metallic sheen |
| *Proteus mirabilis* ATCC® 12453 | Aerobic, 18-24 h @ 33-37°C | Growth, colorless colonies w/ swarming inhibited |
| *Salmonella enterica* serovar Typhimurium ATCC® 14028 | Aerobic, 18-24 h @ 33-37°C | Growth, colorless colonies |
| *Enterococcus faecalis* ATCC® 29212 | Aerobic, 18-24 h @ 33-37°C | Inhibition (partial to complete) |
| *Staphylococcus aureus* ATCC® 25923 | Aerobic, 18-24 h @ 33-37°C | Inhibition (partial to complete) |

## LIMITATIONS
1. Organisms other than gram-negative enteric bacilli can grow on EMB (Levine) Agar.[3]
2. Gram-negative organisms other than *E. coli* can produce a green metallic sheen on EMB (Levine) Agar. Additional biochemical and/or serological testing is required for definitive identification of the test isolate. Consult appropriate references for further instructions.[4,5]
3. Organisms other than gram-negative enteric bacilli can grow on EMB (Levine) Agar.[3]

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## BIBLIOGRAPHY


1. Holt-Harris, J.E. and O. Teague. 1916. J. Infect. Dis. 18:596.
2. Levine, M. 1918. J. Infect. Dis. 23:43.
3. MacFaddin, J.F. 1985. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol. 1. Williams & Wilkins, Baltimore, MD.
4. Food and Drug Administration. 2000. Bacteriological Analytical Manual Online. AOAC International, Gaithersburg, MD. http://www.fda.gov/Food/ScienceResearch LaboratoryMethods/BacteriologicalAnalyticalManual.htm.
5. Downes, F.P. and K. Ito. 2001. Compendium of Methods for the Microbiological Examination of Foods. 4th ed. American Public Health Association, Washington, D.C.


Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, sample collection, storage and transportation, materials required, quality control, and limitations.




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