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 the *Enterobacteriaceae*. 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. The Levine modification of EMB Agar does not contain sucrose, and the lactose concentration is increased.[2] Levine claimed that this formulation provided better differentiation between *Escherichia coli* and *Enterobacter* species.

PRINCIPLE
Eosin and methylene blue dyes provide for differentiation of enteric gram-negative bacilli based the fermentation of lactose and the absorption of the dyes by the bacterial 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, slow lactose-fermenters 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	65.0 mg
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.

PROCEDURE
1. Inoculate and streak the specimen as soon as possible after it is received in the laboratory.
2. If material is being cultured directly from a swab, roll the swab over a small area of the agar surface. Streak the plate for isolation using an inoculating loop.
3. Incubate plate aerobically at 33-37°C for 18-24 hours.
4. Examine plate for typical colony morphology. On EMB (Levine) Agar, colonies of lactose-fermenters are blue-black with a green metallic sheen or pink-brown and mucoid. Nonlactose fermenting colonies are transparent, colorless, or amber.

QUALITY CONTROL
All lot numbers of EMB (Levine) Agar have been tested using the following quality control organisms and have been found to be acceptable. This quality control testing meets or exceeds CLSI standards.[3] Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient 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)

*CLSI recommended organism

LIMITATIONS
1. Gram-negative organisms other than *E. coli* can produce a green metallic sheen on EMB (Levine) Agar. Further testing may be required for definitive identification, following established laboratory procedures.[4]
2. Organisms other than gram-negative enteric bacilli can grow on EMB (Levine) Agar.[4]

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. Clinical and Laboratory Standards Institute (CLSI). 2004. Quality Control for Commercially Prepared Microbiological Culture Media; Approved Standard, 3rd ed. M22-A3. CLSI, Wayne, PA.
4. MacFaddin, J.F. 1985. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol. 1. Williams & Wilkins, Baltimore, MD.

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

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